RESEARCH ON SALB (*Microcyclus ulei*) IN BRAZIL-RECENT PROGRESS[1]

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ABSTRACT

Prior to the establishment of National Rubber and Oil Palm Research Centre (CNPSD) in Manaus, much of the research on SALB was carried out in a somewhat discontinuous and isolated manner. The creation of this specialised crop research centre, under Brazilian Agriculture Research Enterprise (EMBRAPA) in 1975, facilitated a centrally planned research programme, enlarged to include co-operating units in the different rubber growing states, to be instituted in the country.

Commencing initially with 1 full-time pathologist at CNPSD, Manaus, and 2 at CEPLAC, Bahia, work was continued on chemical control up to 1980. By 1982, there were some 10 pathologists engaged on various areas of research on SALB.

As SALB remains a problem of overriding importance on plantations, research continues to stress on its control with chemicals. Other projects currently receiving attention are breeding for resistance, the use of three-part-tree with SALB-resistant crowns, epidemiology studies to develop disease forecasting, zonation planting recommentations involving long term disease surveys and field evaluation for lasting clonal resistance. Work being intensified includes studies on host/parasite relationships, to elucidate the mechanisms for resistance and evolution in the races of the pathogen. Some of the main findings recently obtained are discussed in relation to the current on-going research being pursued on the blight in Brazil.

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INTRODUCTION

Among the South American countries, Brazil has traditionally been closely associated with the production of the para rubber. It was until the 1910s the world's chief supplier of NR, the source being the rubber collected from *Hevea* trees growing naturally in the tropical rainforest.

Interests in increasing this supply through growing of rubber outside the forest therefore were shown early. The Amazônia being the natural home of rubber, attempts by local entrepreneurs in establishing the tree as a plantation crop naturally was started in this tropical region, initially around Manaus, in the country's chief rubber collecting and distributing centre. This was subsequently to follow on a much larger scale by the large American motor tyre companies, such as Ford in Fordlândia and Belterra in Pará State. However, as was well documented, almost all these early plantings ended in failures, the chief cause being identified as the ravages brought about by SALB caused by *Microcyclus ulei*.

As a consequence, early research on rubber did give considerable attention to SALB and its control. Notable works were those by Langford(1943), Albuquerque *et al.* (1972) and Rocha *et al.*(1972/73) on chemical control, Langford(1945) and Camargo *et al.*(1967) on epidemiology, Camargo *et al.*(1967) and Gonçalves *et al.*(1972) on escape areas and Medeiros & Bahia(1967), Gonçalves (1968) and Martins *et al.*(1970) on resistance.

However, a properly established centralised body charged with funding and co-ordinating research by the few rubber researchers scattered in the various states was then non-existent. Partly because of this, many of the valuable research findings relating to SALB were not collated and systematically applied for specific or general field uses on a national scale. In fact, except for only south Bahia where commercial plantations existed was a well-planned programme of research on SALB control carried out from the 1960s at CEPLAC. This was to provide a strong base on which the current satisfactory blight control scheme with fungicides was formulated. In the other equally important areas of research where only fragmentary results were obtained, these were to await the creation in 1975 of the National Rubber and Oil Palm Research Centre (CNPSD) in Manaus, Amazonas State, before an improved and centrally directed programme of research could be instituted.

This paper discusses current research pertaining to SALB in Brazil. with particular reference to recent findings on some of the more important areas of work.

GROWING OF *Microcyclus ulei* IN VITRO

Several researchers (Langford 1945, Blazquez & Owen 1957; Langdon 1966 and Holliday 1970) obtained growth of M. *ulei* in different media. However, the results were generally not satisfactory because of the slow mycelial growth while production of the conidia varied with the media used and was generally sparse. Chee(1978) reported that the fungus grew best at 23°C, produced the most conidia after 3 weeks with optimum germination shown by the younger spores. Langdon (1966) found that isolates sub-cultured continuously for 12 months suffered a drastic reduction in virulency.

For studies involving spore inoculations, a high production of young conidia from a reasonably fast growing culture is essential. This ensures a high inoculum production with a maximum viability. For work on fungal physiology, the choice of a good growth medium used should facilitate the uniform and rapid growth of the fungus. In work relating to clonal resistance studies, one important aspect is the satisfactory preservation of the various isolates or races of the pathogen collected.

A most recent study made at Viçosa Federal University led to the discovery of a new culture medium which greatly improved the isolation, growth and sporulation, and preservation of the pathogen in culture. For isolation, conidia were picked from sporulating lesions from field infected leaflets stored for as long as 15 days after collection. The conidia were transfered to a culture medium made up 6g neopeptone, 1g $MgSO_4.7H_2O$, 2g KH_2PO_4, 10g sucrose, 20g agar. 150 ppm chlorophenicol and 1000*ml* water. Incubation was at 23 - 25°C (Junqueira *et al.* 1984a).

For sub-culturing, fragments of a 15 days old culture were used. The fungus produced, depending on the isolates, an average of 40,000 - 500,000 conidia/mg of dried mycelium when cultured in a medium made up of 6g neopeptone, 1g $MgSO_4.7H_2O$, 2g KH_2PO_4, 10g sucrose or 7g glucose, 20g agar, 2 *ml* "Panvit"(this product can be substituted by 0,25mg threonine + 0,25 mg tryptophan + 10mg lysine hydrochloride), 1000*ml* and pH 5. The culture was incubated at 24°C under alternate light (consisting of 1 h light, 3 h darkness, 1 h light, 3 h darkness, 1 h light and 15 h darkness) using a 40 W fluorescent lamp. The conidia were produced 10-12 days later (Junqueira *et al.* 1984a).

In old cultures, a reduction in conidia production and loss of their viability had been reported (Junqueira *et al.* 1984a). To avoid this, the isolate can best be preserved in a medium made up of 20g sucrose, 2g KH_2PO_4, 1,5 ml of "Panvit" (a product that can be substituted by 0,25mg threonine + 0,25mg tryptophan + 10mg lysine hydrochloride) 10g agar, extract of 250 g potatoes/l and pH 5. Incubation was at 15ºc. Using this medium, it was possible to maintain satisfactorily a continuous fungal growth for up to 90 days or longer, preserving it by repeated sub-culturing (maximum 30 times) without any loss in pathogenicity (Junqueira *et al.* 1984b).

RESISTANCE AND RACES OF *Microcyclus ulei*

The first discovery of resistance in a natural *Hevea* population was reported by Ford researchers, as a result of the failure of the Fordlândia planting in the Pará State, Brazil. Surveying and collecting of resistant materials began in the early 1930s and breeding in 1937. Ford's work was later expanded with the help of the United States Department of Agriculture and the Brazilian Government .Breeding work was done mostly in Brazil at Belterra and Belém (IPEAN-Instituto de Pesquisa Agropecuária do Norte), and also on a smaller scale, at Costa Rica and Guatemala (Holliday 1970).

Several clones were consequently obtained with the desired characteristics. However, the resistance in these selected clones has been reported to have been broken down. This could be due to the development of physiological races or the fact that they were planted in places with environmental conditions different from those where they were selected. Langford (1945) was the first to report the occurrence of races of M.*ulei* attacking resistant clones in Santarém, Pará State. The same worker (1961) also reported a race attacking F 4542 progenies in the Central America. Subsequently, Langdon (1965) identified two races of M.*ulei*, followed by Miller (1966) who identified two more.

Most recently, Junqueira *et al.*(1984c) studied the reactions of 33 rubber clones to 17 isolates of M.*ulei* from several States and Territories in

Brazil where the rubber is cultivated. In this study. the materials tested were clones of *Hevea pauciflora*, *H. benthamiana*, *H. brasiliensis*, poliploides and some oriental clones. It was observed that rubber clones responded differently to infection by several isolates of M. *ulei*, showing that there is a wide variability in pathogenicity. Currently being developed are studies aimed at characterising physiological races of the fungus by protein and isoenzym analyses.

Nothing is known of the resistance mechanism. Blazquez & Owen (1963) , reporting on the histology of infection and spread of the fungus in susceptible and resistant genotypes, noted a hypersensitive reaction. Figari (1965) compared the germination of conidia in aqueous extracts from such genotypes. It was found to be less in those from a resistant clone. He suggested that a toxic phenolic compound was the cause of this effect. The substance detected by Figari was identified by Martins *et al.* (1970) as kampferol-3-rhamnodiglucoside which proved to have a high inhibitory effect on the germination of conidia of M. *ulei*.

In an attempt to follow up this work, CNPSD and the Braunschweig University of West Germany are developing studies together with the following objectives: **a**) establishing cytological and biochemical criteria to select rubber plants resistant to M. *ulei* and **b**) physiological studies on rubber resistance to the different isolates of the pathogen. Currently , field resistance among the newer materials is being evaluated at the Centre in clonal competition trials using the leaf infection scale described by Chee(1976), improved recently by additional criteria such as the number of fallen diseased leaves and % of canopy retained at the end of the disease season. The clones so far evaluated and showing a satisfactory field resistance in Manaus, Amazonas States are: IAN 6158, IAN 6323, Fx 3899 and Fx 4098. Among them, IAN 6158 gives the best yield at the some time (Gonçalves, personal communication, 1983).

Until recently, evaluation of clones for disease resistance and other characteristics was mostly carried out in an isolated manner by researchers in the different states. The information available obviously needed much collating, as was done in a Brazilian Rubber Clone Recomendations Seminar held in 1982 in Brasília. Among the recommendations made, clones for large scale planting by regions are: **a**) for Amazonas and Acre States: IAN 717 and Fx 3864; **b**) Rondônia State: IAN 717, IAN 873 and Fx 3864; **c**) Mato Grosso

State: IAN 717 and IAN 873; **d**) Roraima Territory; IAN 717, IAN 873 and Fx 3864; **e**) Amapá Territory and Pará, Maranhão and North of Goiás States : IAN 717, IAN 3087, Fx 3925, Fx 3899, IAN 6323 and Fx 3810; **f**) São Paulo State: RRIM 600, RRIM 614, RRIM 526, IAN 873, AV 1328, BSA 20, LCB 510, Fx 2261 , Fx 3864, Fx 985 and Fx 25; **g**) Minas Gerais, South Goiás and Mato Grosso do Sul States: RRIM 600, IAN 873, LCB 510 and Fx 3864; **h**) Bahia States: Fx 2261, Fx 985, Fx 3864, Fx 3844 and Fx 4163; **i**) Espírito Santo and Rio de Janeiro States: Fx 25, Fx 2261, Fx 985, Fx 3864, Fx 3846 and IAN 873 (Resultados ... 1983).

EPIDEMIOLOGY AND DISEASE/WEATHER RELATIONSHIPS

According to Langford (1945), Hilton (1955) and Tollenaar (1959), spores of M. *ulei* require a period of at least 10 consecutive hours of high relative humidity of above 95% and high temperature, with the optimum average daily values between 24^{O}c and 26^{O}c, to germinate and infect the host. The conditions most favourable to an occurrence of SALB, according to studies made in the field in Planalto Paulista (São Paulo State) by Camargo *et al.* (1967), and in Bahia State by Rocha & Vasconcelos Filho (1979), are when the air relative humidity is above 95% consecutively for 10 hours during a minimum period of 12 nights per month.

Other conditions favourable to SALB infection are the size and the density of plantation, and the rain distribution pattern. At a farm in the Rio Branco municipality (Acre State), it was observed that a day with light rain during the period of leaflet susceptibility was sufficient to cause an epidemic (Gasparotto *et al.*, 1984a).

The spread of spores, mainly the conidia, occurs through rain water and wind within a plantation. Holliday (1970), Chee (1976a) and Rocha & Vasconcelos Filho (1979) using spore traps, observed that maximum conidia liberation occurred during the day when the relative humidity fell and temperature rose. The number of ascospores, on the other hand, increased during the night to a maximum at 6.00h and decreased to a low level during the day.

Current work on epidemiology in progress in Amazonas, Bahia, Pará and Rondônia States involves the collection of data on local climate (rainfall maximum temperature and wetness period and SALB incidence (based on leaf infection scale by Chee (1976a) number of fallen diseased leaves and % of canopy retained at the end of the disease season).

CONTROL OF SALB

Disease Escape Area

A disease escape area can be defined as one in which the environmental conditions are unfavourable to the development of M. *ulei* but within which rubber can grow and yield satisfactorily.

In a preliminary study (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA 1979), it was suggested that under the Humid Tropical conditions, a region would be considered as a SALB escape area if there occurs therein an annual moisture deficit of 200-350mm, distributed over a period of 4-6 months, with the wintering of the rubber tree occurring preferably in the 3 intermediate months within this period. Within these general criteria, further studies need to be carried out, eg. to determine the exact length of the period when free water is continuously present on the leaflets, in an area considered to be an escape. This represents a factor more important in characterising if a area is unfavourable to the occurrence of M. *ulei* in an epiphytotic form (Gasparotto*et al.* 1984a). Based on current knowledge, the areas that could be considered as being capable of escaping SALB are the litoral areas that experience a constant sea breeze, not permitting long period of wetness on the leaf that favours spore germination and infection, areas bordering large rivers where the relative humidity of the air is normally low, or in places where the wetness duration is not very prolonged (Bastos & Diniz 1980). Rands (1924) earlier observed that, for this reason the rubber plantations in the East Coast of Trinidad, near the sea, suffered little infection by M.*ulei* .

In some areas where rubber is already grown, it was shown clearly that, the conditions for disease escape resulted from unfavourable weather conditions. This is the case of Açailândia municipality (Maranhão State) were *Hevea* has been planted for more than 10 years and is yielding normally without being affected by SALB, despite the presence of fungus in the crowded nursery. This locality has a dry season lasting for more than 4 months and the trees winter within this period (Pinheiro *et al.* 1982a).

Other places considered to be an escape zone wherein rubber plantations are being established are the litoral of Guarapari municipality (Espírito Santo State) and Ituberá litoral (Bahia State) (Trindade & Lim 1982). Camargo *et al.* (1975) observed that in São Paulo State, SALB occurred only in low and badly drained terrains exposed to long-lasting dew conditions. No infection was observed on rubber trees growing upland or in other well--drained areas of the plateau. It was concluded that the growing of rubber might be feasible and free from leaf blight even for the most susceptible clones under the sub-tropical conditions of the São Paulo plateau and similar regions in the neighbouring states.

On the SALB-free São Paulo plateau, Cardoso & Carretero (1982) subsequently reported that a number of clones including RRIM 614, RRIM 600 and LCB 510, produced an average of 1,300kg of dry rubber/ha/year. The well-known Tira-Teima plantation on the litoral in Espírito Santo State (Rodrigues 1982) similary produced a satisfactory yield, with a mixture of local and oriental clones, averaging 25.80g of rubber/tree/tapping. In the Maranhão State, a 12-year-old experimental planting of mixed Brazilian clones, located in SALB-free Açailândia also gave high yield, the best estimated at an equivalent of 2,000kg of dry rubber/ha/year (Pinheiro *et al.* 1982b).

Crown Budding and SALB

Cramer pioneer of the crown budding in rubber, developed the idea of building the ideal three-component rubber tree (Radjino 1969). Maas in 1934, suggested top-budding a high-yielding clone with LCB 870 to protect against *Oidium* attack. In Java, however, this method was not commercially successful. Yield depression caused by LCB 870 crown was found to be greater than that caused by the disease (Radjino 1969).

Langford (1957) reported that crown budding in South America began in 1940. In commercial practice, high-yielding eastern clones were used with *Microcyclus*-resistant crowns. However, a similar depression effect of crown budding on the yield of panel clones was reported in almost all recent papers on the subject (Radjino 1969, Tan & Leong 1976, Leong & Yoon 1976 and Bahia & Sena Gomes 1981). In the light of this, Moraes (1982) recently suggested that there was a need for research on the type of changes to panel clone capable of affecting its resultant yield, such as reduction in the number of latex rings, increase in blockage indices, obstruction to translocation in tissues at the point between crown and panel union or a reduction in the efficiency of latex synthesis.

The same author studied the number of rings of 10 panel clones under 5 different crowns, in comparison with non-crown-budded tree. In all the panels clones. the number of rings under the crown of F 4512 was significantly superior in relation to others. Under the crown of F 4512, all panels showed a bigger trunk girthing rate. The panel bark tissues with this crown clone showed a bigger number of rings. He therefore concluded that **a**) crown budding exerted a morphogenetic effect on the differentiation of laticiferous rings in the panel clone, probably independent of the growth rate of the stem; **b**) canopy clone with the characteristics already identified for vigour and disease resistance, together with the character of a high number of laticiferous rings, can provide the ideal type of a high yielding rubber three-part-tree.

Lion *et al.* (1982), working in the Amazônica region, obtained the best results from trials conducted in the 1960s using the crown clone PA 31 on IAN 713, IAN 717, Fx 3810, Fx 3864 and Fx 3899 as panels. The three-part-tree yielded an average of 35.56g of dried rubber/tree/tapping, compared with 12.58g of dried rubber/tree/tapping in the non-crown-budded tree, showing no depression effect. Pinheiro *et al.* (1982b), also obtained similar good results with crown budding. The latter researchers were able to suggest, pending more trials, that crown budding in the meantime be recommended to plantations for small scale planting. In the Bahia State, on the other hand, crown budding trials did not give the same good results (Dunham *et al.* 1982 , Maia 1982 and Sena Gomes *et al.* 1982).

In a recent Rubber Crown Budding Seminar held in 1982 in Brasília, it was decided that, for the Amazônia region, the following crown clones could be recommended: PA 31, IAN 6486, IAN 7388, Fx 4049 and Fx 636. For south Bahia, the crown clones were PA 31, Px and IAN 6545. The choice of panel clones was left to local planters to choose, from those that are already available (Tecnologia... 1982).

Artificial Defoliation

Control of SALB is normally expensive because of repeated rounds of fungicide treatment needed until refoliation is completed and advanced to a stage when the leaflets are no more susceptible to infection. This becomes even more expensive when wintering on trees in some clones is not uniform and tended to be prolonged.

The use of artificial defoliation is to reduce and uniformize the wintering period of the trees, in order to facilitate the aplication of fungicides and to reduce the number of spraying or fogging rounds.

The process of annual wintering is set in motion by a series of physiological changes in the foliage, commencing much before the earliest symptoms are observed (Chua 1970). Rao (1972) showed it was possible by chemical defoliation to advance and shorten the wintering period. Rao & Azaldin(1973), subsequently suggested that an application of a chemical defoliant a couple of weeks in advance of wintering might hasten this process and bring about a rapid and complete defoliation. In Malaysia, this technique has been adopted commercially to avoid leaf fall caused by *Oidium* and *Colletotrichum*. In Brazil, Romano *et al.* (1982) also tried fogging the heat-stable defoliant thidiazuron (Lim 1982) in a 13 years old field of Fx 3899 and Fx 25. Satisfactory results were shown only for Fx 3899, the technique obviously requiring further research. Santos & Pereira (1984) are currently screening a chemical mixture made up of ethephon + copper sulphate + boric acid as possible leaf defoliant. When tested in a clonal nursery, 90% of plants stayed leafless for 7 days after the treatment. But at the concentration used, some injuries were caused to the young shoots of the plants. In general, the plants showed a rapid and uniform refoliation soon after. The next stage will be to test the promising mixture on adult trees.

Chemical Control

For SALB control, the fungicides currently being recommended are mancozeb (Manço 1968), benomyl (Albuquerque *et al.* 1972 and Rocha *et al.* 1972/73), methyl thiophanate (Rocha *et al.* 1978), triadimephon (Silva 1979) carbendazin and triforine (Gasparotto *et al.* 1984b). In nurseries and clonal gardens, the selected fungicides are applied weekly during the wet season and at two weekly intervals during the dry season. On mature trees on plantations, these are applied weekly during the refoliation period.

In south Bahia, aerial spraying of effective water-based fungicides against SALB employed up to a total of 7 treatment rounds per disease season per year (Bezerra *et al.* 1982). From 1981, when estate-scale treatment with oil-based fungicides by ground fogging with Leco 120 D was introduced, up to 10 weekly fogging rounds were recommended (Corrêa *et al.* 1984).

In the Amazônia. the few new plantations established in the 1970s suffered the worst effect of SALB. this normally in combination with poor soil and weed management. Gasparotto *et al.* (1984c) demonstrated that an integrated approach to SALB control, combining fungicidal treatment by fogging or spraying with improved weed control and adequate manuring, achieved the best results under such a situation. Details pertaining to more recent developments in SALB chemical control is given in a separate paper entitled "Recent progress in fungicide application technology for rubber leaf disease control" presented at this same workshop.

CONCLUDING REMARKS

Brazil is currently rapidly expanding its huge programme of rubber planting planned and initiated under the PROBOR projects(Barros *et al.* 1983). Delayed for nearly a century by SALB in doing so earlier, this major constraint to the domestication of *Hevea* in the New World had been shown in more recent research to be not insurmountable. In this brief review highlighting the more significant aspects of work on SALB and its control in Brazil, evidence suggests that rubber may be grown as a profitable commercial crop, given the attention it deserves in continuous research as has been initiated in Brazil (Lim *et al.* 1984).

In the so-called traditional areas of rubber cultivation in the Amazônia and south Bahia, the hot, humid tropical weather provokes the most virulent attacks by M. *ulei* on the tree host. In south Bahia, where plantations barely survived from years of continuous attacks, large-scale government-sponsored aerial spraying under PROMASE during the last decade (Bezerra *et al.* 1982), based on the findings of research derived from the 1960-70s, gave a satisfactory rehabilitation of this rubber(Souza *et al.* 1982). In the Amazô nia where new plantations were being established in the presence of SALB and other major leaf diseases, their effect made worse by poor soil and weed management. fungicidal treatment should preferably be integrated with improved manuring and weed control, to give the desired benefit, as suggested by Gasparotto *et al.* (1984c). With the objective of minimising treatment costs, a multi-disciplinary approach in current research by agricultural engineers and pathologists led to a rapid demonstration that a ground-based fogger (Leco 120 D or a locally made Jacto machine), when suitably adapted and provided with the correct minimum fungicide dosages, is an efficient and more practical applicator than a helicopter, for dispensing fungicides or insecticides to plantations. Parallel to these, some of the most recent research results, suggesting the existance of newer SALB-resistant clones, more potent fungicides, newer SALB escape areas and improved methods for culturing *M.ulei*, show satisfactory progress is being achieved with a well co-ordinated approach to research on SALB, as directed by CNPSD from Manaus.

On the on-going research and those being intensified, those relating to zonation clonal planting recommendations will have a considerable bearing on the well-being or health of the new rubber due for planting under PROBOR III. Although yet fully defined in terms of precise criteria for SALB prevalence of otherwise, available data do suggest the existence in Brazil of vast areas of possible SALB escape zones. It is generally felt that the real potential for rapid rubber growing lies in these areas.

In areas where SALB cannot be avoided, on the other hand, a more discriminate use of clones with observed stable field resistance, similar to that used under Enviromax Principle in Malaysia, holds the most promise. Economic control of SALB with protective fungicides having been demonstrated, use of this new technology to reduce SALB damage to existing plantations is now readily available. Other major problems relating to mechanisms in disease resistance, loss of resistance in clones and the emergence of new races of the pathogen, currently also being studied, are in line with the objective of eventually developing an overall, truly integrated approach to solving the many problems still posed by SALB in Brazil, or in the other rubber growing countries of the New World.

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