INTERNATIONAL RUBBER RESEARCH AND DEVELOPMENT BOARD

PROPOSALS FOR THE INTERNATIONAL NET-WORK RESEARCH PROGRAMME ON TAPPING PANEL DRYNESS

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1. PREAMBLE

Tapping Panel Dryness (TPD), commonly known as brown bast, is a syndrome encountered in rubber plantations, characterised by spontaneous drying up of the tapping cut resulting in abnormally low yield or stoppage of latex production. The disorder was reported for the first time in Brazil in 1887 in wild *Hevea* trees in the Amazon forest and at the beginning of the century in plantations in Asia (Rutgers and Dammerman, 1914).

1.1 *Symptoms*

TPD symptoms range from partial dryness with no browning of the tapping cut, browning and thickening of the bark to cracking and deformation of the bark in some instances. The syndrome is characterised by the appearance of tylosoids and the coagulation of latex *in situ* (de Fay, 1981; de Fay and Hebant, 1980; Paranjothy *et al.* 1976), abnormal behaviour of parenchyma cells adjoining the laticifers and general increase in synthesis of polyphenols (Rands, 1921). A detailed review of the histological, histochemical and cytological study of the affected bark has been presented by de Fay and Jacob (1989).

1.2 *Causative organisms*

The involvement of a causative organism in TPD was suspected by early workers (Keuchenius, 1924; Rands, 1921; Sharples, 1922). But these workers were unable to demonstrate the existence of an agent responsible for causing tapping panel dryness. Later, the possibility of pathogenic causes for certain types of cortical necrosis which leads to stoppage of flow was reported by Nandris *et al.* (1984), Peries and Brochier (1965) and Zheng Guanbiao *et al.* (1982). Though rickettsia-like organisms (RLO) was implicated by Zheng Guanbioa *et al.* (1988), no confirmatory evidence could so far be obtained. This aspect needs a thorough re-investigation utilising modern methodology.

1.3 *Soil, climatic and clonal characters in relation to TPD*

Influence of climate and growth period on the incidence of brown bast disease was reported by early workers (Harmsen, 1919; Vollema, 1949; Compagnon *et al.* 1953; Bealing and Chua, 1972). Through the analysis of soil, leaves and latex, the effect of unbalanced nutrition favouring the incidence of disease was reported by Pushpadas *et al.* (1975). Clonal sensitivity to tapping panel dryness was observed by many workers (Bangham and d'Agremond, 1939; Dijkman, 1951; Heusser and Holder, 1930; Ostendorf, 1941; Vollema and Dijkman, 1939).

1.4 *Biochemical and physiological changes*

A variety of biochemical and biophysical changes take place at the different stages of this syndrome. The most common symptoms include a phase of excessive late dripping of latex and a simultaneous fall in the rubber content and after a period of time, gradual decline in the volume per tapping. Colloidal stability of the latex also get reduced resulting in particle damage, flocculation of rubber particles

in situ, and early plugging of latex vessels (Chrestin *et al.* 1984). A reduction in turgor pressure (Sethuraj *et al.* 1977), change in latex flow pattern (Sethuraj, 1968) and a sharp increase in bursting index (Eschbach *et al.* 1983) were also found associated with TPD.

According to Chua (1967) the reserves of starch and carbohydrates are not depleted. Interestingly, de Fay (1981) reported abundance of starch grains in the wood of affected trees and the vascular rays were found to function normally.

Existence of an endogenous NAD(P)H oxidase in lutoids which generates toxic forms of oxygen, responsible for the peroxidase degradation of organelle membranes in the latex from affected trees was reported by many workers (Chrestin, 1984; Chrestin *et al.* 1984; Chrestin, 1985; Cretin and Bangratz, 1983). Simultaneously, decrease in concentrations of latex cytosol scavangers (reduced thiols and ascrobate) as well as virtual disappearance of scavenging enzyme activities (SOD and catalase) was reported (Chrestin, 1984, 1985). The combination of increased peroxidative activities and considerably diminished quantities of scavengers in latex from affected trees result in destabilisation and lysis of lutoids leading to coagulation (Chrestin, 1989). A possible damage to all the membrane structures in laticifers and resulting impairment of nutrient supply and water exchange at plasmalemma was suggested by Chai Kim Chun *et al.* (1969) and Pushpadas *et al.* (1975).

High intensity of exploitation is known to promote incidence of tapping panel dryness in plantations; the proportion of dry trees increases with tapping intensity, particularly the frequency (Bealing and Chua, 1972; Chua, 1967; Paranjothy *et al.* 1976). Intensive exploitation is reported to result in excessive outflow of latex and consequent nutritional stress (Chua, 1967; Schweizer, 1949; Sharples and Lambourne, 1924; Taylor, 1926), inadequate organic resources (Chua, 1966; Tupy, 1984), and Cu and K deficiency (Compagnon *et al.* 1953). Changes in mineral ratios, especially K_2O/CaO and Mg/P, were reported by Beaufils (1957). An increase in K content and K/Ca and K/P ratios in latex was observed by Pushpadas *et al.* (1975).

Certain forms of bark dryness are transitory and do not display the characteristic symptoms of the formation of tylosoids or activation of the phenolic metabolism (de Fay and Jacob, 1989). Traumatism resulting from tapping, chemical application or pathological infection can cause formation of ethylene (Yeang and Pratt, 1978) and its influence in biochemical, anatomical and histological parameters is known (Liebermann, 1973). Over stimulation (dose and frequency) or over tapping can lead to excessive endogenous ethylene production and deleterious effects on cellular systems (Chrestin, 1984, 1985). Induction of bark dryness through deliberate over stimulation with ethrel results in imbalance in peroxidase activities and consequent disorganisation of the membrane structures. This may lead to the onset of bark dryness.

According to Eschbach *et al.* (1986) a reduction in sucrose, thiol and Mg contents and increase in redox potential (RP) are connected with high incidence of bark dryness. Reduced availability of assimilates and the essential enzyme systems may be the principal cause of more frequent occurrence of the disease.

Incidence of TPD can be reduced by reducing the exploitation intensity. Tapping rest imposed for varying periods may revive some of the trees, but in majority of cases, re-occurrence of the syndrome is encountered.

Recent thinking centres round the question why only certain percentage of trees in a monoclonal population get affected. Genetics of root stock has been implicated and this aspect will receive adequate attention in the international network research programme envisaged by the International Rubber Research and Development Board (Sethuraj, 1989).

2. IRRDB INITIATIVE FOR AN INTERNATIONAL RESEARCH PROGRAMME ON TPD

With the release of many precocious high yielding clones from different member Research Institutes of the IRRDB, the concern for TPD increased considerably because most of the high yielding clones evolved were found to be highly susceptible to TPD syndrome. Fifteen to twenty percent dry trees in about five years of tapping has become a common feature in many plantations and there were also cases where the incidence was over 30%. This disturbing situation became a focus of attention during technical discussions in IRRDB. It has been projected that the global loss in production of natural rubber due to TPD with an average 10% incidence would be as high as 5,00,000 tonnes, which in value is equivalent to US$ 400 million. Eight decades of research on TPD by different institutes neither could reveal the cause of this syndrome nor could suggest any effective preventive or curative treatment. The situation being so, the priority assigned to research on TPD by different member institutes of IRRDB at present is totally inadequate.

The Physiology Group of the IRRDB convened a Symposium on TPD in Penang in June 1989. Further discussions took place in the Physiology Group meetings of the IRRDB in Kunming, China in October 1990 and in Manila, Philippines in October 1991. The need to organise an international research programme to pool the expertise and facilities existing in the IRRDB Institutes was realised and the Board nominated me (Dr. M.R. Sethuraj, Director, Rubber Research Institute of India) as the Team Leader to formulate and coordinate an international research programme in its Manila meeting. I visited the RRIM, SCATC and IRCA and held detailed discussions with the Core Groups of scientists working in the area of TPD. This was done with the idea to formulate an international research programme with four major centres of research: RRII, RRIM, SCATC and IRCA with provision for scientists from other member institutes of the IRRDB to get attached to one of these centres for varying periods.

3. SUMMARY OF DISCUSSIONS

The present knowledge base and results of current experiments were reviewed. Theoretical analysis of the possible causes and strategies for management was made in these discussions. The constraints, both in terms of logistics in conducting experiments and in technical competence in certain specialised areas were identified. The discussions also brought out the fact that some of the sophisticated and modern approaches required may be outside the present competence of the IRRDB Institutes and it would be prudent to identify external centres of excellence for assigning such work on contract basis. The salient points of these discussions were presented to the Secretary, IRRDB and he was appraised of the need for some initial IRRDB funding to start an international network research programme. This will form Phase I of the project proposals. Phase II would involve investigations utilising most modern methodological approaches and molecular level interventions. Such studies will necessarily be collaborative projects between IRRDB Institutes and recognised outside centres of excellence where expertise and facilities are available to undertake studies at such level.

Taking stock of the expertise and infrastructure available with the IRRDB member Institutes, four rubber research institutes were identified as main centres. These are IRCA, RRII, RRIM and SCATC. Scientists from the other IRRDB Institutes will be given opportunity for attachment to these centres for varying periods.

3.1 *At RRIM, Malaysia*

I had discussions with various Groups at the Rubber Research Institute of Malaysia on 27th and 28th April, 1992. On 27th I had detailed discussions with the Leader and Members of the project team on TPD. This project team was formed in 1990, comprising inter-departmental researchers and has already conducted a study to assess the physiological parameters under various exploitation treatments aimed at inducing dryness. The results are being analysed.

The team is now engaged in conducting comprehensive studies from an experiment laid out in an estate. The treatments in the experiment include: 1/2S d/3, RRIMFLOW, 1/2S d/3 with stimulation and 1/2S d/2. The concept of target yield is followed in this experiment. The clone studied is PB 260.

In addition to the above, experiments covering density of planting and nutritional levels are also conducted for studying different aspects of TPD.

Some new approaches suggested to the team were:

* Individual tree approach for comparisons.
* Field comparisons when differences in the incidence of TPD between fields are significantly high.
* Strengthening of multi-disciplinary team approach, and the emphasis on using the same samples for such studies.
* Need for reinvestigation on the involvement of a pathogen using modern immunological techniques (possibility of contracting this work to any well equipped competent international or national laboratories is to be explored).
* Studies on the possible role of genetic variability of the root stock on the development of TPD syndrome in the scion in a monoclonal population.
* Possibility of mass multiplying identified 'resistant root stock'. This can be attempted through tissue culture or rooting of cuttings and experiments using such genetically identical root stock may be taken up.
* Genetic mapping of such root stocks.

The Group was of the opinion that a correct definition of TPD is necessary to ensure uniformity in the syndrome under study in various centres. The Group also agreed that work at molecular level should be initiated, as only then many unresolved fundamental questions related to the onset of TPD would be answered. It was also agreed that the IRRDB should suggest structured common experiments for all the four centres. The IRRDB may try to give some seed money to each Institute to acquire facilities for strengthening the logistic capability for conducting the experiments strictly as per schedule on a regular basis.

In conclusion it was stated that all theoretical and practical approaches should be explored, all leads are worth looking for obtaining clues (even certain observations by the farmers may be critically assessed). International funding to strengthen the logistic capability of the Institutes, for properly conducting the experiments as per strict schedule, initiating research at molecular level and also to assign contract work to recognised centres of excellence in specialised areas should be sought. The IRRDB Physiologists Group should discuss and agree upon common structured programme to be conducted in all the four centres and the methodology to be followed should be standardised. Special emphasis on single tree approach as well as on studies of the root stock should be given. A re-investigation on the possible involvement of a pathogen should also be made.

I had detailed discussions with the Members of the Industry on 28th April 1992. En. Chai Chan Tat, En. Ong Tee San, En. Gan Liong Tiang, En. Teoh Cheng Hai, En. Santhana Kumar and En. Shamsuri represented the natural rubber industry in Malaysia. Detailed review of the experiences in the industry was made. Relationship of TPD with clones, nutrient levels, topography, exploitation systems, etc. was critically examined and it was generally agreed that no definite pattern normally emerges except for the fact that there is clonal variation in regard to susceptibility and that the incidence of TPD is always higher with intensive exploitation. The possible relationship between genotypic variability of the root stocks in a field with the incidence of TPD was extensively discussed. It was made clear that the industry can cooperate with the Institute (RRIM) in carrying out large scale trials using genetically identical root stocks developed either by tissue culture or rooted cuttings. It was also opined that we should also simultaneously develop methods of management for TPD. Various potential approaches including simple agronomic

methods like higher intensity of planting, skipping tapping in excessive late dripping trees on alternate days of tapping etc. were suggested and the industry again expressed willingness to cooperate with the Institute and the IRRDB for carrying out such studies and to record observations.

I had discussions with the Director and Asst. Director, RRIM. They were in full agreement on the need to have an international network research programme on TPD. They also shared the views and concerns expressed in the Group Meetings.

3.2. *At SCATC, China*

I had discussions at SCATC on 30th April and 1st and 2nd May 1992 with the Core Group of Scientists working on TPD. The following points emerged from the discussions.

The researchers of SCATC classified TPD mainly into two types:

(a) Inner bark dryness: The inner bark, ie. that part of the bark which is adjacent to the cambium becomes necrotic or brown first, while the outer bark can still produce latex normally. The necrotic part however, may expand very quickly and invade large areas of panel and it seems that this type of dryness can hardly be cured.

(b) Outer bark dyrness: The dryness of the bark starts at the outer bark first, and gradually expand to the inner part of the bark. This kind of symptom can be treated by surgical removal of the affected part and application of curative chemcials.

Cause of TPD: Most of the researchers in SCATC consider that TPD is caused by over exploitation or over stimulation. They however consider that stress of any kind like typhoons, root disease and other mechancial shock may also induce TPD.

Some researchers suspect a pathogenic agent (RLO or MLO). Results of an experiment indicate that the buds from trees with fasciations will lead to TPD when they are budded to healthy plants and allowed to grow into budded trees. It has been suggested that the pathogen that leads to TPD is an RLO. It was however evident that Koch's postulates were not followed in these studies and therefore the results are not very convincing. The rate of TPD in the field is about 10-15%, while the rate of switch broom disease in the field is only 0.4% indicating lack of any strong relationship between the two. A model for the incidence of TPD involving various physiological and biochemical stages was presented by the Researchers of SCATC. The model integrates views expressed by different IRRDB member institutes and can be of much use at the time of formulation of an international programme.

Treatment of TPD trees: The bark under the tapping cut of the TPD tree is first segmented into several vertical bark columns by making vertical grooves on the bark, upto the bottom of the trunk, and then the bark is peeled, column by column. Due care is given to ensure that the cambium is not damaged. After the bark under the cut is peeled off, the exposed surface is pasted with petrolatum. It takes 7-8 years of renewal before it becomes ready for exploitation. During that stage, puncture tapping is done at high panel.

Based on the discussions it was concluded that: The observations and experiments on TPD described above show that much more work should be done both to elucidate the mechanism of TPD and to develop methods of treatment. Research work in the future should be done with more planning and scientific input. We should meticulously describe the process of TPD generation, and study the various factors that are related to various stages of TPD. Fundamental studies at cellular and molecular level should be strengthened. A comprehensive reinvestigation of any pathogenic involvement using Koch's postulates must be attempted. Further, anatomical studies must go parallel with the physiological studies.

3.3 *At IRCA, France*

I held discussions with the Core Group of Scientists of IRCA and Prof. d'Auzac on 5th and 6th June, 1992. The main points emerged are summarised below:

The necessity for investigations on the role of root stock on the incidence of TPD was stressed by me. The theoretical aspects of this view were explained to the Group and they have in general accepted this idea. It was also stated by them that the vigour of the tree depends to a great extent on the success of grafting and that a degree of incompetability may cause biological disorders which may be related to this syndrome. It was felt that much research is needed to verify this hypothesis. In this respect, tissue culture should make it possible to multiply clonal grafting stock and thus to measure its influence. If a root stock genotype is identified which can render some resistance to the scion from the onset of this syndrome, then it can be mass multiplied and used as root stock for susceptible clones. It was also pointed that the root system and its environment (composition, structure, soil water availability) may also play a role in the appearance of bark dryness. It was also agreed that it is necessary to collect samples on a single tree basis rather than on a field basis to get correct information on comparative biochemistry and physiology of affected and normal trees. The Group generally agreed to the suggestion that studies on ethylene metabolism in response to tapping should find an important place in the total programme. The Group was interested in learning the Chinese finding that the trees affected by TPD can be classified as (a) Inner brown bast (b) Outer brown bast. The Group also showed interest in the treatment procedures adopted in China.

J.L. Jacob reminded the meeting of IRCA's position, presented at the meeting on brown bast held by RRIM in Penang in 1989. The term 'brown bast' covers different phenomena which are all finally expressed by dryness of the tapping cut. This results in confusion in symptomatological analysis, complicating research on the problem. 'Reversible brown bast' may be observed. This depends on the season and may also be caused by a certain fatigue of laticiferous tissues caused by over-intensive exploitation. In the latter case, reducing tapping intensity or stimulation intensity can significantly reduce the phenomenon, which remains limited to the tapped area of the bark. Biochemically, it is shown by a decrease in the hydrocarbon reserves of latex, activation of the systems generating toxic oxygen and decrease of the activity of the enzymes involved in intracellular anti-senescence mechanisms. This results in local thrombosis in the latex production system caused by *in situ* bursting of lutoids; this causes *in situ* coagulation and the appearance and spread of bark dryness. The other type(s) of brown bast are related to tissue necrosis involving not only the laticifers but also surrounding tissues in the phloem. This irreversible type spreads at varying rates along the whole trunk. In addition, it is not necessarily related to the tapping cut and does not always start there. Its symptomatology is different in histology and cytology and also in analysis of the biochemical functioning of the laticiferous tissues. The phenomenon is induced by stress related to soil structure or composition; stress related to drought or stress caused by physiological fatigue. In the latter case, reversible brown bast may be a prior state to irreversible brown bast. In other words, the inducing agent may be over-tapping in the long term. The oncogenic nature of the phenomena observed *in situ* (especially tylosoids and their anarchic multiplication) and the frequently non-random distribution of diseased rubber trees lead to suspecting a presence of a pathogen (virus, viroid, mycoplasma, rickettsia) in spite of failure to date to identify any such organism in situ. Molecular biology can be valuable aid in solving this problem.

From a practical point of view, although there is no effective cure so far (bark scraping, panel excision etc. are not satisfactory), unsuitable exploitation of clones leading to fatigue and finally to irreversible necrosis should be avoided. Latex diagnosis can be extremely useful. In addition, the planting of sensitive clones (with a low threshold for the induction of brown bast phenomena) should be avoided in situations where ecoclimatic stress may be considerable.

3.4 *Discussion with the Secretary, IRRDB*

Summary of discussions with the Secretary, IRRDB on 8th June is given below:

Programme should be based on the deployment of IRRDB experts on carefully planned experiments, with some IRRDB seed money to cover extra costs. In addition, it is essential to use outside centres of excellence for specific items of work at the molecular/cell level using immunological techniques. Overall, to get this programme properly worked out and operated, the difficult problem of logistics needs to be tackled; some IRRDB Institutes have cost problems associated with, for example, provision of vehicles, fuel etc.

The general plan will be to combine

* experiments in a few countries, with a different clone in each country
* use of the full range of physiological and biochemical tests
* use of outside experts for techniques which are outside IRRDB expertise

The main components of the common project will be:

(a) Field Experiments

Experiment I. Survey of existing monoclonal fields, marking individual trees as completely dry/partially dry/normal. Select small groups comprising normal (five trees), partially dry (five trees) and dry (five trees) for comparative studies on biochemistry, physiology, soil etc.

Experiment II. Monoclonal field divided up for different exploitation systems (1/2S d/1 to 1/2S d/7). Record all parameters (physiological, biochemical) every 15 days: observations on onset of TPD symptoms.

Experiment III. Monoclonal field with normal exploitation system. Case history of trees from Day 1 of opening. Record all parameters once every 2-3 weeks and monitor symptoms of onset of TPD.

(b) Fundamental studies to establish the role of root stock, if any

(c) Effect of generally uniform root stock (produced through tissue culture or rooting of cuttings) on incidence of TPD in different clones.

(d) Experiments to cope with existing fields with high incidence of TPD

This includes (i) experiments on management methods (chemicals, change of panel, tapping rest during stress period etc.), and (ii) investigation of novel exploitation systems.

(e) Search for pathogen, using appropriate laboratory, eg. NRI (UK), Advanced Centre of Virology (New Delhi), University of Malaysia, IRCA.

Note: When TPD is detected, the symptoms must be recorded in a descriptive manner, eg. 'partially-dry - no colour' 'dry, brown ring' etc.

(f) Projects on investigations at molecular level manipulations will form Phase II of the project proposals and international centres of excellence will have to be identified for collaboration.

The Secretary, IRRDB requested that I should make a presentation of Project proposals for International net-work Research Programme on TPD at the next meeting of IRRDB at Jakarta.

4.1 *The concept*

The fact that the TPD incidence in rubber remains an unresolved problem in spite of eight decades of research in various centres is suggestive of the complexity of this disorder. Earlier work has discounted the possibility of any pathogenic involvement. Failure to isolate any organism, absence of any usual symptom associated with MLO, RLO, virus or viroids, the random pattern of occurrence of TPD in plantations and absence of any sign of infectivity through tapping knife etc. strengthen the earlier contention that no pathogen is involved. Although it has generally been described as a physiological disorder, no abiotic factor either could be firmly established as the cause for the onset of this syndrome. Clonal variations in the rate of incidence has been established indicating that the proneness to this syndrome is perhaps genetically determined. At the same time it is intriguing why only certain percentage of trees succumb to this syndrome when all the plants in a monoclonal population are genetically homogeneous and are subjected to the same stress and exploitation levels. The various biochemical changes reported to be associated with the onset of this syndrome could be the consequence rather than the cause *per se* of TPD. The possible random distribution of varying genotypes of the root stock, originating from seeds, perhaps present a parallel to the randomness of the occurrence of TPD. Factors related to root activity and metabolism may vary from plant to plant as every root stock is different from the other genotypically. This aspect has not been studied in detail.

A comprehensive analysis of the factors which predisposes the onset of this syndrome; of the physiological and biochemical changes associated, and of any genetic character of the root stock that may contribute towards development of this syndrome may give us some useful leads and future approaches in research aimed at various methods for the management of this disorder can be formulated. The IRRDB initiative to pool the resources, expertise and facilities of member institutes of IRRDB to address this problem can yield results only if properly planned structured experiments, both in the field and in the laboratory are carried out in different centres; such division of work and experiments having been decided upon by the level of specialisation in each centre. The studies should address a variety of questions and would range from basic field experiments to most sophisticated fundamental studies at molecular level. Certain aspects of such studies would be outside the present competence of the member institutes of IRRDB and it would be prudent to identify outside 'centres of excellence' to assign such work on a contract basis rather than to build up the expertise and facilities in IRRDB institutes.

4.2 *Investigations proposed*

The proposed studies can be divided into the following aspects.

4.2.1 Phase 1

4.2.1.1 Structured field experiments in four IRRDB institutes: IRCA, RRII, RRIM and SCATC.

4.2.1.2 Fundamental investigations related to the question of influence of root stock. (Part of this programme can be carried out at the IRRDB centres and certain aspects such as genetic mapping of root stocks can be assigned to an identified outside centre of excellence).

4.2.1.3 Experiment to evaluate the effect of genetically uniform root stock on TPD in different clones.

4.2.1.4 Prophylactic and curative methods of TPD management

4.2.1.5 A reinvestigation into the possible role of any pathogenic agent. (To be assigned to a selected external centre of excellence).

4.2.2 Phase II

4.2.2.1 Identification of genes coding for enzymes involved in ethylene synthesis in response to tapping and molecular intervention to regulate the rate of synthesis.

4.2.2.2 Identification of genomic 'anti-TPD factors' and molecular level manipulations to evolve transgenic plants incorporating such factors.

4.2.2.3 Molecular level investigations on key enzymes involved in biosynthesis of rubber and their relationship, if any, with TPD.

4.3 *Essential Details of Experiments*

4.3.1 General aspects

Some amount of commonality should be ensured among the experiments conducted at various centres. Every Institute should try to follow uniformity in:

* Definition and description of the syndrome
* Technical terms used
* Methodology followed for analysis of commonly selected parameters.
* Frequency of observation.
* Statistical methods of analysis, etc.

It is now evident that there are different kinds of TPD with varying symptoms. There may be practical difficulties in selecting a particular symptom alone, because these varying symptoms are vaguely described and there is some amount of confusion. For example, TPD has been classified as

* Reversible type
* Irreversible type
* Inner brown bast
* Outer brown bast
* Panel dryness always preceeded by excessive late dripping
* Panel dryness not necessarily preceded by any late dripping
* Bark dryness followed by abnormal oncogenous growth.

A practical approach would be to succinctly describe these symptoms tree-wise and categorise. The experimenters may come across different categories in the same field and a correct record of these symptoms of TPD may become highly useful for later analysis of the data. In all the common experiments envisaged, observations will be tree-based rather than field-based because such an approach is necessary considering the unpredictability and randomness of its occurrence.

4.3.2 Experiments under Phase I

4.3.2.1 Structured field experiments in IRRDB Institutes

Experiment I

Physiological and biochemical parameters in normal, partially dry and completely dry trees

Objective: To detect if there is any consistant variations in physiological and biochemical paratmeters associated with TPD.

Experimental approach

All trees in a monoclonal population should be critically examined and categorised as normal, partially dry and completely dry. Further descriptions (inner brown bast, outer brown bast , with late dripping, without late dripping, etc) can also be attempted and recorded. Groups of three to five trees from these three main categories distributed in a compact area can be taken as a 'unit' of study for comparative purposes. Physiological , biochemical and anatomical parameters may be studied in latex, bark, roots, leaves and soil etc. as indicated in Table I. The Institutes are free to add more parameters based on facilities and expertise. These comparative studies may be repeated for many such units comprising the three categories, ie. normal, partially dry and dry trees, in different fields and for different clones. The data may be analysed using appropriate statistical methods to find out if there are significant differences among these categories in terms of any parameter.

Experiment II

Changes in physiological parameters before the onset of the TPD syndrome

Objective: To monitor changes in physiological and biochemical parameters in individual trees in a monoclonal population at periodic intervals and to associate such changes with the onset of TPD syndrome.

Experimental approach

200 normal trees of a known high yielding susceptible clone (suggested - India, RRII 105; Malaysia, PB 260: IRCA, PB 235 and China, RRIM 600) may be selected from a field in its first month of tapping. The trees may be monitored for the onset of any symptom associated with TPD at regular intervals (fortnightly). Physiological and biochemical parameters may also be monitored at regular intervals on a tree basis and case histories maintained. The number of trees to be selected at random for each parameter is to be decided by the complexity and time requirement for estimation of each of these parameters. Table 2 indicates a pattern which can be followed. Once an incipient TPD syndrome is detected the pattern of change, if any, of the parameters prior to the onset should be critically examined. After substantial number of trees acquire TPD syndrome, a critical evaluation is to be made to find out if there is any commnonality in the pattern of changes of parameters before the onset of TPD syndrome.

Table 1. Parameters to be recorded.

S.No.	Latex*	Bark		Leaves	Roots	Soil in the tree basin
		Scion	Stock			
1.	Initial flow rate	1. Anatomical &histoch-emical inve-stigations	1. Anatomical &histoch-emical inve-stigations	Total nutrients N,P,K,Ca, Mg,Mn,Cu	1. Total nutrients N,P,K,Ca Mg,Mn,Cu	1.CEC 2.Avaiable nutrient status for macro and micro nutrients
2.	Total volume					
3.	Plugging index					
4.	Dry rubber content %					
5.	Pretapping turgor pressure	2. Starch 3. Sucrose	2. Lipid peroxi-dation		2. Root CEC 3. Isozyme analysis	
6.	Total solids	4. Total sol-uble pro-tein	3. SOD			
7.	Bursting index	5. Total nut-rients N,P, K,Ca,Mg, Mn,Cu.B,Zn	4. Cytokinins 5. ABA			
8.	Sucrose (Serum)					
9.	Thiols (Serum)					
10.	Pi (Serum)					
11.	Mg (Serum)	6. Lipid perox-dation				
12.	K (Serum)	7. SOD				
13.	Membrane lipids					
14.	Lipid peroxi-dase	8.Cytokinins				
15.	SOD					
16.	Total nutrients (from total solids)P,K,Ca, Mg,Mn,Cu,B, Zn	9. ABA				

*only for partially dry and normal trees.

Table 2. Case history of trees - parameters to be recorded.

Sl. No.	Parameter	No. of trees to be covered	Frequency of recording
1.	Initial flow rate	200 Nos.	15days
2.	Total volume	200 Nos.	15 days
3.	Plugging index	200 Nos.	15 days
4.	D.R.C.	200 Nos.	15 days
5.	TPD Scoring (visual)	200 Nos.	15 days
6.	Sucrose (latex)	100 Nos.	15 days
7.	Thiols	100 Nos.	15 days
8.	Pi	100 Nos.	15 days
9.	Mg++	100 Nos.	15 days
10.	K	100 Nos.	15 days
11.	Leaf: Total nutrients P, K,Ca,Mg,Mn,Cu,B,Zn	100 Nos.	Once in the season
12.	Bursting index	50 Nos.	15 days
13.	Membrane lipids	50 Nos.	15 days
14.	Membrane proteins by electrophoresis	50 Nos.	15 days
15.	Lipid peroxidase SOD activity	50 Nos.	15 days
16.	Soil: Total nutrients	50 Nos.	Once in the season
17.	Root: Total nutrients	50 Nos.	Once in the season
18.	Latex: Total nutrients	50 Nos.	Monthly

Experiment III

Effect of different intensities of Tapping on physiological parameters of latex and bark and their relationship with the onset of dryness.

Objective: To elucidate the role of tapping intensity in promoting the incidence of tapping panel dryness.

Experimental approach

A statistically laid out experiment may be started to compare different intensities of tapping. The treatments suggested are: d/1, d/2, d/3 and d/7; number of replications 6. The trees may be observed for any syndrome of TPD once in a fortnight. A few trees at random may be selected from each plot for physiological observations. The following parameters may be recorded.

1. Yield (on every tapping day)
2. drc (on every fortnight)
3. Plugging index (on every fortnight)
4. Bursting index (on every fortnight)
5. Sucrose (on every fortnight)
6. Pi (on every fortnight)
7. Thiols (on every fortnight)
8. SOD (on every fortnight)
9. Turgor pressure of the bark (on every fortnight)

In addition to the above, other useful and relevant parameters can also be studied by the Institutes depending on the facilities and expertise available. Once symptoms of TPD appear, extensive physiological and biological studies both of the scion and stock may be carried out. Descriptive characterisation of the symptoms of individual trees may be attempted.

4.3.2.2 Fundamental investigations related to the question of influence of root stock

Objective: To ascertain whether genotypic variation in the root stock and the resultant variations in root activity have any role in predisposing the scion to the incidence of TPD.

Experimental approach

A direct approach by attempting genetic mapping of the root stock as well as an indirect approach by recording parameters in the scion budded on to "resistant" and "tolerant" genotypes of the root stock are suggested. A susceptible precocious high yielding clone is to be subjected to intensive tapping. The incidence of TPD may be recorded at regular periodic intervals. (If such records are already available the suggested studies can be conducted in that field).

Classification of root stock as "tolerant" and "susceptible" in terms of the time taken for the syndrome to appear may be made. The suggested studies should be conducted on individual rootstock plants within each group. Intra-group and inter-group comparisons should be made.

(a) Genetic mapping

RAPD, RFLP mapping or Isozyme analysis.

RAPD markers have been shown to be highly useful in the construction of genetic maps and many researchers now prefer this approach compared to RFLP mapping. It is strongly suggested that member institutes having facilities may initiate studies in this direction. Collaboration with renowned national institutes having expertise and experience in this technique may also be encouraged to reduce the time lag to acquire the necessary expertise and facilities in individual institutes.

Isozyme analysis:

The Institutes which have already acquired sufficient expertise in isozyme analysis may extend such studies to the root stocks of 'tolerant' and 'susceptible' groups.

Any common factors among the individuals in a group or contrasting factors between the two groups may give us some clues of possible 'predisposing factors' or 'anti-TPD factors'. Generation of such fundamental information is necessary for any meaningful conceptual development required for Phase II of the project proposals.

(b) Development of methods for generation of plantlets from the identified root stock for multiplication.

The identified root stocks will have to be multiplied by first inducing regeneration by removal of scion plant and then multiply the genotype either through tissue culture methods or rooting of cuttings. Even before this is achieved, high yielding normal genotypes may be identified from polyclonal seedling populations from an area of high incidence of TPD and methods perfected to multiply such plants either through tissue culture or rooting of cuttings assuming 'genetic tolerance' to TPD in such selected genotypes.

(c) Indirect methods to assess the influence or root stock

The levels of cytokinins and ABA may be quantified in latex and bark of individual trees from the above two groups. Attempts may also be made to collect xylem sap for such measurements as it would be an ideal material to study the influence of root stock interaction. A method may also be perfected for *in situ* measurement of ethylene generation in the bark. Intra-group and inter-group comparisons of these parameters may reveal some pattern which will become useful for planning Phase II experiments.

4.3.2.3 Effect of genetically uniform root stock on the incidence of TPD in different clones.

Objective: To ascertain whether incidence of TPD can be controlled by using genetically uniform 'TPD resistant' root stock.

Experimental approach

Once the method for mass multiplication of an identified genotype of root stock is perfected, either through tissue culture or rooting of cuttings, these materials may be used as root stocks in a statistically laid out experiment employing three clones. Root stock raised from genetically heterozygous seeds will

be used as control. The experiment would involve six treatments; with two types of root stock (homozygous and heterozygous) and three clones. The design suggested is RBD with four replications. The experiment can be repeated with different genotypes of root stocks. Incidence of TPD should be monitored at regular intervals.

4.3.2.4 Experiments on TPD management

Investigations on different prophylactic and curative methods may be carried out.The methods chosen can include experiments founded on scientifically sound assumptions, experiments based on observations and experience of planters and other emperical approaches. Each institute may be given the freedom to design such experiments. However, certain basic principles of approach can be enumerated.

* Novel methods of exploitation (eg. RRIMFLOW).
* Reduction in tapping intensity for trees showing late dripping and partial dryness (reversible type) by avoiding tapping on every alternate tapping day.
* Application of chemicals aimed at reducing the generation of ethylene.
* Application of chemicals with anti-senescence properties.
* Surgical treatments for removing the affected parts followed by application of chemicals to promote renewal of bark.
* Other emperical methods based on observations and experience.

It is suggested that the number of treatments and number of replications may be determined in such a manner that the data can be analysed statistically.

4.3.2.5 A reinvestigation into the possible role of any pathogenic agent

Objective: To investigate the possible association of any biotic agent(s) with TPD, employing modern biochemical immunological and molecular methods.

Experimental approach

Search for any possible association of biotic agents such as fungi, bacteria, virus, viroids, MLO, RLO, BLO and phytomonas will be made employing the most modern and appropriate immunological and molecular techniques. Such studies will also be supported by standard techniques (EM, field testing).

As most modern methods and approaches are to be employed, such aspects of study will have to be assigned to a recognised external Institute of excellence (two project proposals; one from Natural Resources Institute, UK and another from Advanced Centre of Virology, IARI, New Delhi have been received. There is substantial difference in the level of funding sought for by these two institutes. A critical evaluation of the project proposals is required to take a decision).

4.3.3 Experiments under Phase II

The relevance of the Projects listed under Phase II would depend on the results of related experiments listed under Phase I. The conceptual foundations of these projects rests on some of the assumptions made in planning the Phase I experiments. Basically, these projects aim at genomic interventions to instil anti-TPD characteristics and to evolve transgenic plants incorporating such attributes.

The member institutes of IRRDB are not, at present, adequately equipped in terms of facilities and skill, to take up all aspects of these projects. It would, therefore, be prudent to carefully identify Laboratories/Centres with proven competence to carry out experiments involving latest techniques in plant molecular biology and genetic engineering and assign these projects to these laboratories on contract. Certain IRRDB member Institutes (IRCA, RRII, RRIM) however, have perfected the protocol for regeneration of plants from callus through somatic embryogenesis. Experiments with protoplast culture also are under way. Therefore, the suggested projects can be collaborative projects between IRRDB Laboratories and outside Centres of excellence.

4.3.3.1 Identification of genes coding for enzymes involved in ethylene synthesis in response to tapping and molecular intervention to regulate the rate of synthesis.

The concept

Abnormal endogenous synthesis of ethylene as a result of tapping injury is implicated with the incidence of TPD. Many of the biochemical changes at cellular and sub-cellular level, reported to be associated with different phases of TPD have been reported to be associated with the action of ethylene *in vivo*. This abnormal and excessive production of ethylene may have similiarities with the climacteric synthesis of ethylene during the senescence of cells as well as during ripening. The approach suggested is to identify the genes coding for enzymes such as ACC Synthase and ACC Oxidase and to construct antisense RNA, which can reduce the expression of the concerned genes in a gene dosage-dependent manner. The methodology to be followed will be similar to that reported by Hamilton, *et al* (1990) in their work involving antisense technology in tomato.

The details of experimental approach and methodology can be decided only after discussion with the scientists of the laboratory identified as a 'Centre of Excellence' by IRRDB for assigning this project.

4.3.3.2 Identification of genomic 'anti-TPD factors' and molecular level manipulations to evolve transgenic plants incorporating such factors.

Objective: To evolve transgenic plants incorporating genomic 'anti-TPD factors'.

The concept

Any distinct polymorphism in DNA between the 'TPD-susceptible' group of root stocks and 'TPD-resistant' group of root stocks obtained from RFLP/RAPD analysis (high level of sequence divergence based on results of Phase I Experiment No. 4.2.1.2) would indicate genomic involvement in either promoting or preventing the development of TPD in the scion. Theoretically it should be possible to identify the relevant DNA fragments and to use it for identification and cloning of the gene encoding enzymes related to 'anti-TPD factor'. With the rapid progress that is being made in plant transgenic technology, an objective to design a transgenic plant of rubber incorporating such character is within the realm of possibility.

The details of experimental approach and methodology can be decided only after discussion with the scientists of the laboratory identified as a 'Centre of Excellence' by IRRDB for assigning this project.

4.3.3.3 Molecular level investigations on key enzymes involved in biosynthesis of rubber and their relationship, if any, with TPD.

Objective: To characterise genomic clones encoding important enzymes in the rubber biosynthesis pathways and to examine if there is any abberation at genomic level in TPD-affected plants.

The Concept

Characterisation of cDNA genomic clones encoding 3-hydroxy-3-methylglutaryl-coenzyme A reductase from *Hevea brasiliensis* have already been achieved (Chye *et al.*, 1991). Fundamental investigations at molecular level to elucidate the relationship, if any, between TPD and such genomic abberations affecting the efficiency of rubber biosynthesis would be rewarding.

The details of experimental approach and methodology can be decided only after discussion with the scientists of the laboratory identified as a 'Centre of Excellence' by IRRDB for assigning this project.

5. NEED FOR EXTERNAL FUNDING AND FINANCIAL OUTLAY

The four IRRDB member Institutes, selected as Centres, have adequate facilities and personnel to implement most of the projects listed under Phase I. However, certain practical constraints have been identified during the discussions I had in these centres. Regular recording of a number of parameters strictly as per the schedule is a prerequisite for the success of these experiments. For eg., the trees go dry at random and spontaneously and if the recordings of parameters in the weeks prior to the onset of the syndrome have not been done due to constraints, the very purpose of the experiment, to establish relationship between changes in certain parameters with the development of TPD Syndrome will be jeopardised. Some funding to these centres would improve their logistic capability. (Eg., To provide a vehicle with running expenses exclusively for the purpose). The quantum of laboratory analysis involved is tremendous and the centres would need hiring of laboratory and field staff on a temporary basis for these Projects. Provision should also be made for training/attachment of Scientists from other IRRDB Institutes in these centres for varying periods as well as for international travel for monitoring and discussions.

In view of the above, the four centres should be provided with modest funding to meet their immediate requirements. Provision should also be made for contract work with recognised outside centres of excellence to carry out certain aspects of the Project envisaged (Causative organism, genetic mapping of root stocks). As there can considerable time lag in obtaining any international funding, it is strongly suggested that IRRDB should try to raise this fund, and considering the economic importance of the Project it would be a step in the right direction.

5.1 Proposed budget for Phase I

A model budget is given in Table 3.

Table 3. Proposed Budget

(IN US DOLLARS)

	1993	1994	1995
Contribution to four IRRDB Member Institutions (RRII, RRIM, IRCA & SCATC)	1,20,000 (30,000x4)	60,000 (15,000x4)	60,000 (15,000x4)
Contract Research:-			
A. Pathogen aspects	35,000	15,000	10,000
B. Molecular aspects	15,000	10,000	10,000
Attachment of trainees		25,000	20,000
International travel		5,000	10,000
Total	170,000	115,000	110,000

Total for three years = US$ 395,000

5.2 Funding for Phase II

International funding will be however necessary for the Projects under Phase II and the IRRDB should take appropriate steps. These Projects may have to be assigned to selected centres of excellence and some such laboratories are likely to be located in Europe or USA. These Project proposals can be formulated only after in-depth discussion with the concerned scientists of these selected laboratories.

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