Developments in Plantation Crops Research, 1998, pp. xi-xiv.

KEYNOTE ADDRESS

BIOTECHNOLOGY IN PLANTATION CROPS RESEARCH

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Biotechnology is going to be the most important tool in agriculture during the first quarter of the 21st century. Mass multiplication of desirable genotypes can be achieved through micropropagation techniques. Modifications with a specific and desirable character can be achieved through molecular intervention. However, these approaches should be resorted to only when it is economical or has distinctive advantages over conventional methods. Tissue culture is a tool through which a biotechnological innovation can be achieved.

Recalcitrance in most tree crops is a factor which increases cost of production of tissue culture plants. Economic considerations should form the basis for resorting to tissue culture techniques. For example, although a repeatable protocol for shoot tip culture of rubber has been developed, it could not be commercialised due to its prohibitive cost. But, if the tissue culture plant nullifies the root stock effect thereby increasing the yield by say 20%, the economic gains over a period of the life span of the crop may be several fold the additional initial investment.

Somatic embryogenesis is another pathway for propagation. Although this method is much easy, uniformity of progeny is seldom achieved. However, the somaclonal variants produced may be utilised for selection of a desirable trait. Anther culture and protoplast culture are other techniques. Though protoplast culture is difficult, it is advantageous in transformation and somatic hybridisation. The prime concern in adopting any of these techniques should be the economic advantage.

Molecular intervention

The molecular approaches offer the potential to make relatively quick and specific changes in any cultivar without disrupting their otherwise desirable genetic constitution. However, there are several technical constraints in developing a transgenic plant. But if we are able to develop a single transgenic plant with a stable and agronomically desirable and functional gene, further propagation can be done within a short period by conventional vegetative means.

The strategic decision in adoption of molecular biological means should be based on (a) an acceptable cost for transformation and regeneration compared to its expected economic return and (b) possibility of obtaining the target genotype by manipulation of one or a few genes only. For example, if a gene for resistance to a disease could be identified in a wild variety of the crop plant, and if a feasible protocol for isolation of the target gene is available, it is worth investing in it. An understanding of the regulation of expression of the target gene and the methods for achieving adequate spatial and temporal control are also essential. Temporal and tissue specific promoters are to be isolated and put to use to achieve such control. It is likely that an introduced gene instead of amplifying an existing pathway, would silence it. But there are alternative methods to achieve the targeted objective. Although attention was drawn towards molecular biology for the past fifteen years, commercial biotechnology made a beginning only in 1995, with the production of genetically engineered tomatoes with an antisense gene for delaying the ripening, thus enhancing the keeping quality. This was followed by development of a few herbicide resistant crop plants in USA and cotton with Bt gene in Australia. It is expected that a large number of transgenic crop plants will soon be commercially released in USA, Europe, China and Australia.

The important areas in which biotechnological approaches can be tried are resistance to diseases, pests and nematodes, herbicide resistance, tolerance to abiotic stresses like drought, cold, etc. and for regulation of metabolic pathways. The regulation of metabolic pathway can be attempted in cases where more than one product is formed from the same pathway. In such cases amplification of specific genes responsible for specific enzyme activity could lead to production of other specific products from the same pathway. A particular pathway can be stopped if desired, using antisense genes.

In sustainable agriculture, we aim at reduction in the use of chemical fertilizers. This could be achieved by using genetic engineering. Amplification of the activity of the carrier protein responsible for nutrient uptake and transport can lead to more efficient nutrient uptake, thus reducing the nutrient input.

Rubber plant is one in which there is wide scope for molecular farming. The non-rubber contents of latex like inositols, proteins, aminoacids, etc. can be converted into high value secondary products, if genes responsible for specific enzymes could be introduced into the system.

Several attempts have been made to induce disease resistance in crop plants through genetic transformation. A gene responsible for production of phytoalexin from grapewine has been introduced into tobacco plants to induce resistance to *Botrytis* sp. Introduction of genes encoding for polypeptides with inhibitory effect on fungal enzymes have been attempted. Transfer of genes responsible for pathogen induced resistance genes have been tried in potato. The introduction of Bt gene in cotton is too well known. The economic viability of the use of bacculo viruses in integrated pest management can be improved through molecular intervention. Genes responsible for resistance to root-knot and cyst nematodes have been identified in certain plants. These genes could impart nematode resistance in a transgenic crop.

The areas in which molecular approaches are to be attempted vary from crop to crop. In pepper resistance to *Phytophthora* appears to be an important area. In tea, quality improvement can be attempted through molecular intervention. In coffee, resistance to berry borer needs such attempts. In cardamom, katte disease resistance should be aimed at. The development of efficient and reliable protocols for plant regeneration is essential in crops like coconut and oil palm before genetic transformation is attempted. Every lab with minimum facility can work on RAPD, RFLP and AFLP analysis and also develop cDNA and genomic libraries.

Molecular intervention in rubber

Initially attempts were made to study the genetic variability in rubber through isozyme profiles. Later RFLP analyses were attempted. Now RAPD analysis is being carried out in three centres, *viz*. Rubber Research Institutes of India and Malaysia and at CIRAD in France for genetic analysis of *Hevea*. An estimation of phylogenic relationship from mitochondrial and ribosomal DNA RFLPs have also been attempted. Measurement of genetic distances through RAPD analysis and identification of certain traits by block analysis are other areas attempted. There are several potential areas for molecular intervention in *Hevea*. Laticifer specific gene expression in *Hevea* has been demonstrated by scientists working in Singapore. Wound induced accumulation of mRNA combing a hevein sequence in laticiferous tissue, cloning of the gene encoding for HMG Co A - a key enzyme in rubber biosynthesis and nucleotide sequencing of the gene for rubber chain elongation factor have been accomplished in USA (Research on guayule, an alternative for *Hevea* in rubber production is actively undertaken in USA. The wide propaganda on allergy due to *Hevea* rubber in the West has to be viewed in this context). Molecular cloning, characterisation and expression of genes responsible for production of SOD has been accomplished in *Hevea*. SOD is an anti-ageing enzyme responsible for scavenging free radicals.

Transformation systems have been developed for *Hevea*. The Rubber Research Institute of India (RRII) has perfected two protocols for transformation - *Agrobacterium* mediated and other by using a gene gun.

Tapping panel dryness is a serious disorder in rubber in which trees go dry during the economic yielding phase. It is estimated that the global annual loss due to this disorder is US$ 900 million per year. Molecular analysis of the genotypes showing tolerance to this disorder has been attempted at the RRII. The RAPD profiles indicated the presence of two polymorphic bands in the tolerant plants while they were absent in the susceptible. In China, DNA polymorphism between tolerant and susceptible *Hevea* genotypes for *Oidium* disease has been observed.

The strategies identified for molecular approach for mitigation of TPD effect are, (a) amplification of MnSOD, (b) regulation of *in vivo* ethylene production using antisense ACC synthase, and (c) promotion of endogenous cytokinin production by introduction of an ipt gene. The constructs of the genes have already been made. Introduction of these into *Hevea* genome to produce a transgenic plant is being attempted. If we could achieve a 20 per cent increase in productivity through use of a transgenic plant, the incremental yield from one lakh hectares over a period of 20 years is expected to give a return of US$ 780 million.

Intellectual property rights

There exists some differences in the rules relating to patents prevalent in India and those stipulated in the World Trade Organisation (WTO) agreement which our country has recently signed. While the WTO provides for product patents, in India only process patents are given. WTO grants patents for any invention - products or process - in all fields of technology, provided they are new, involve inventive steps and are capable of industrial applications, but provide flexibility for extension in areas like diagnostic, therapeutic, surgical, biological process for production of plants, animals, etc. We have to develop our own system for providing patents in these areas.

Another area in which WTO provides patent is microorganisms. The definition of microorganism in this case is confusing and so the changes incorporated by gene manipulation could some-times be interpreted as microbial intervention as some of these genes come from microbes. In contrast, the Indian patent laws do not allow patenting of live forms, though patents based on microbial process are permitted. WTO also requires patent protection of plant varieties. There is no such patent protection in India at present. Another area is the difference in duration of patent coverage - 20 year uniform coverage is provided under WTO while Indian system provides only 7 years for food and pharmaceuticals.

There have been claims in the US for a share of profits from a commercialised transgenic apple variety with Bt gene by a company which holds the patents for the promoter of the gene construct. But in plantation crops where release of a variety takes more than 20 years, the question of patents may not arise.

The patent rights provided under WTO will not take away the farmers' and researchers' rights. Even now we use many patented materials in our research. Similarly, the farmer will not be prevented from using patented seeds for producing a crop for his use. Only when he goes in for commercial production of seeds the question of patent arises. Therefore, the impact of biotechnology on seed production need not scare anyone.

Biosafety measures

In countries like USA, UK, Japan and Australia, specific biosafety rules have been framed for evaluation of transgenic plants. In India, such rules are yet to be evolved. In USA, although a permit system was initially introduced it was later replaced by notification system. Rules in this area are available with USDA and Biotechnology Board of UK which can provide basic guidelines for evolving such rules in our country.

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Samsuddin, Z., Tan, H. and Yoon, P.K. 1985. Variations, heritabilities and correlations of photosynthetic rates, yield and vigour in young Hevea seedling progenies. Int. Rubb. Conf. 1985, Kuala Lumpur, Malaysia.

Saraswathyamma, C.K. and Sethuraj, M.R. 1975. Clonal variation in latex flow characteristic and yield in the rubber. J. Plant. Crops., 3: 14-15.

Satheesan, K.V., Rao, G.G. and Sethuraj, M.R. 1982. The osmotic concentration and dilution of B and C-sera in latex of Hevea brasiliensis in relation to rubber yield. Proceedings of PLACROSYM V, in press.

Satheesan, K.V., Rao, G.G., Sethuraj, M.R. and Raghavendra, A.S. 1984. Canopy photosynthesis in rubber (Hevea brasiliensis): characteristics of leaves in relation to light interception. Advances in photosynthesis research Vol. IV. (ed.) Sybesma, C., Martinus Nijhoff/W. Junk, pp. 348-351.

Satheesan, K.V., Rao, G.G. and Sethuraj, M.R. 1986. Studies on the light climate in relation to planting density in fully foliated rubber plantations. Proc. TREEPHYSINDIA 82: in press.

Sethuraj, M.R. 1968. Studies on the physiological aspects of rubber production. I. Theoretical consideration and preliminary observation. Rubber Board Bull., 9: 47-62.

Sethuraj, M.R. 1981. Yield components in Hevea brasiliensis - theoretical considerations. Plant Cell. Environ., 1981, 4: 81-83.

Sethuraj, M.R. 1984. Internation rubber research programme in physiology and exploitation. Proc. IRRDB. Coll. Expl. Physiol. Am. Hevea, Montpellier, pp. 357-366.

Sethuraj, M.R. 1985. Physiology of growth and yield in Hevea brasiliensis. Int. Rubb. Conf., 1985, Kuala Lumpur.

Sethuraj, M.R. and George, M.J. 1976. Drainage area of the bark and soil moisture content as factors influencing latex flow in Hevea brasiliensis. Indian J. Plant. Physiol., 19: 12-14.

Sethuraj, M.R. and Raghavendra, A.S. 1984. The pattern of latex flow from rubber tree Hevea brasiliensis in relation to water stress. J. Cell. Biochem., Suppl. 8B: 236.

Sethuraj, M.R., Subronto, Sulochanamma, S. and Subbarayalu, G. 1978. Two indices to quantify latex flow characteristics in Hevea brasiliensis. Muell. Arg. Indian J. Agric. Sci., 48: 521-524.

Sherif, P.M. and Sethuraj, M.R. 1978. The role of lipids and proteins in the mechanism of latex vessel plugging in Hevea brasiliensis. Physiol. Plant., 42: 351-353.

Shorrocks, V.M. 1965. Mineral nutrition, growth, and nutrient cycle of Hevea brasiliensis. I. Growth and nutrition content. J. Rubb. Res. Inst. Malays., 19: 32-47.

Simmonds, N.W. 1982. Some ideas on botanical research on rubber. Trop. Agric., (Trin.), 59: 1-8.

Sivanadyan, K., Haridas, G. and Pushparajah, E. 1975. Reduced immaturity period of Hevea brasiliensis. Proc. Int. Rubb. Conf. Kuala Lumpur, 3: 147-157.

Southorn, W.A. 1969. Physiology of Hevea (latex flow). J. Rubb. Res. Inst.

Malaya, 21: 494-512.

Southorn, W.A. and Edwin, E.E. 1968. Latex flow studies. II. Influence of lutoids on the stability and flow of Hevea latex. J. Rubb. Res. Inst. Malaya, 20: 187-200.

Southorn, W.A. and Gomez, J.B. 1970. Latex flow studies. VII. Influence of length of tapping cut on latex flow pattern. J. Rubb. Res. Inst. Malaya, 23: 15-22.

Southorn, W.A. and Yip, E. 1968. Latex flow studies. III. Electrostatic considerations in the colloidal stability of fresh Hevea latex. J. Rubb. Res. Inst. Malaya, 20: 201-215.

Templeton, J.K. 1968. Growth studies in Hevea brasiliensis. I. Growth analysis up to seven years after budgrafting. J. Rubb. Res. Inst. Malaya, 20: 136-146.

Templeton, J.K. 1969. Partition of assimilates. J. Rubb. Res. Inst. Malaya, 21: 259-263.

Tupy, J. 1980. Modification of pH of latex cytoplasm by ethylene. Phytochemistry, 19: 509-511.

Tupy, J. 1985. Some aspects of sucrose transport and utilization in latex producing bark of Hevea brasiliensis Muell. Arg. Biol. Plant., 27: 51-64.

Waistie, R.L. 1965. The occurrence of an endogenous type of endotrophic mycorrhiza in Hevea brasiliensis. Trans. Br. Mycol. Soc., 48: 167-178.

Westgarth, D.R. and Buttery, B.R. 1965. The effect of density of planting on growth, yield and economic exploitation of Hevea brasiliensis. I. The effect on growth and yield. J. Rubb. Res. Inst. Malaya, 1965, 19: 62.

Yeang, H.Y. and Daud, M.N. 1984. Quantification of latex vessel plugging by the intensity of plugging. J. Rubb. Res. Inst. Malaysia, 32: 164-169.

Yeang, H.Y., Jacob, J.L., Privot, J.C. and Vidal, A. 1986. Invertase activity in Hevea latex serum: Interaction between pH and serum concentration. J. Nat. Rubb. Res., 1: 16-24.

Yip, E. and Gomez, P.B. 1984. Characterization of cell sap of Hevea and its influence on cessation of latex flow. J. Rubb. Res. Inst. Malaysia, 32: 1-19.

Yip, E., Southorn, W.A. and Gomez, J.B. 1974. Latex flow studies. IX. Effects of application of yield stimulants on rheology of Hevea latex and on concentrations of charged components in its sera. J. Rubb. Res. Inst. Malaysia, 24: 103-110.

Zhou Zhongyu, -Yuan Xichui, Wei Lizhen, Guo Quiyan, Huang Xiang, Zhan Sairong, Chen Chuanquin and Liu Jinxing. 1983. Approaches to early predicting mature rubber yield potential at juvenile stage and thir theoretical basis. IRRDB Sci. Symp. China, 1983. Preprint.

Gomez, J.B. and Moir, G.F.J. 1979. The ultracytology of latex vessels in Hevea brasiliensis. Monograph No. 4, Malaysian Rubb. Res. Dev. Board, Kaula Lumpur.

Gooding, E.G.B. 1952. Studies in the physiology of latex. II. Effects of various factors on the concentration of latex of Hevea brasiliensis. New Phytol., 51: 11-29.

Hălle, F. and Martin, R. 1968. Etude de la croissance rhythmique chez l'Hevea (Hevea brasiliensis Muell. Arg. Euphorbia-cees-crotonidees). Adansonia [N.S.], 8 (h): 475-503.

Ho, C.Y., Subramaniam, A. and Yong, W.M. 1975. Lipids associated with the particles in Hevea latex. Proc. Int. Rubb. Conf. Kuala Lumpur 1975, Vol. II, pp. 441-456.

IRRDB. 1984. Compte-Rendu du Colloque Exploitation - Physiologie et Amelioration de l'Hevea. Intl. Rubb. Res. Dev. Board and Inst. Rech. Caout., Montpellier, France.

Lim, C.M., Southorn, W.A., Gomez, J.B., and Yip, E. 1969. Electrophysiological phenomena in Hevea brasiliensis. J. Rubb. Res. Inst. Malaya, 21: 524-542.

Low, F.C. and Gomez, J.B. 1984. Carbohydrate status of exploited Hevea. III. Non structural carbohydrates in the bark. J. Rubb. Res. Inst. Malaysia, 32: 82-102.

Lustinec, J. and Resing, W.L. 1965. Methodes pour la delimitation de l'aire drainee a l'aide des microsaignees et des radioisotopes. Rev. Gen. Caout. Plast., 42: 1161-1165.

Lustinec, J., Chai, K.C. and Resing, W.L. 1966. L'aire drainee chez les jeunes arbres de l'Hevea brasiliensis. Rev. Gen. Caout. Plast, 43: 1343-1354.

Lustinec, J., Simmer, J. and Resing, W.L. 1969. Trunk contraction of Hevea. Biol. Plant., 11: 236-244.

Lynen, F. 1969. Biochemical problems of rubber synthesis. J. Rubb. Res. Inst. Malaya, 21: 389-406.

Macrae, S., Gilliland, M.G. and Van Staden, J. 1986. Rubber production in guayule: Determination of rubber producing potential. Plant Physiol., 81: 1027-1032.

Milford, G.F.J., Paardekooper, E.C. and Ho, C.Y. 1969. Latex vessel plugging: its importace to yield and clonal behaviour. J. Rubb. Res. Inst. Malaya, 21: 274-282.

Moraes, V.H.F. 1977. Rubber. In: Ecophysiology of Tropical Crops. (eds.) Alvim, P. de T. and Kozlowski, T.T., Academic Press, New York, pp. 315-331.

National Academy of Sciences. 1977. Guayule: An alternative source of Natural Rubber. Natl. Acad. Sciences, Washington, 80 pp.

Ng, E.K., P'Ng, T.C. and Lee, C.K. 1970. Tapping systems for young rubber. Planters Bull. Rubb. Res. Inst. Malaya, 111: 285-315.

Ninane, F. 1967. Evapotranspiration reelle et croissance de jeunes heveas soumis a differentes humidites du sol. Rev. Gen. Caout. Plast., 44: 207-212.

Ong, S.H. 1978. Problems associated with Hevea breeding in RRIM. Malay. Appl. Biol., 6: 171.

Paardekooper, E.C. and Samosorn, S. 1969. Clonal variation in latex flow patterns. J. Rubb. Res. Inst. Malaya, 21: 264-273.

Paardekooper, E.C. and Sookmark, S. 1969. Diurnal variation in latex yield J. Rubb. Res. Inst. Malaya, 21: 341-347.

Pakianathan, S.W., Boatman, S.G. and Taysum, D.H. 1966. Particle aggregation following dilution of Hevea latex: A possible mechanism for the closure of latex vessels after tapping. J. Rubb. Res. Inst. Malaya, 19: 259-271.

Pakianathan, S.W., Wain, R.L. and Ng, E.K. 1975. Studies on displacement area on tapping in mature Hevea trees. Proc. Int. Rubb. Conf. 1975. Vol. II: 225.

Pillay, P.N.R. 1980. (ed.) Handbook of Natural Rubber Production in India. Rubber Research Institute of India, Kottayam, Kerala, India. 668 pp.

Premakumari, D., Sherif, P.M. and Sethuraj, M.R. 1980. Variations in lutoid stability and rubber particle stability as factors influencing yield depression during drought in Hevea brasiliensis. J. Plant. Crops, 1980, 8: 43-47.

Pujarniscle, S. 1970. Etude biochemique des lutoides du latex d'Hevea brasiliensis difference et analogie avec les lysosomes. Rev. Gen. Caout. Plast., 47: 175-178.

Raghavendra, A.S., Sulochanamma, S., Rao, G.G., Mathew, S., Satheesan, K.V. and Sethuraj, M.R. 1984. The pattern of latex flow in relation to clonal variation, plugging and drought tolerance. In: Proc. IRRDB Colloquium Expl. Physiol. Am. Hevea, Montpellier, July 1984, pp. 205-226.

Rands, R.D. 1921. Brown Bast disease of plantation rubber, its cause and prevention. Archf. Rubbercult. Ned.-Indie., 5: 233-271.

Rao, G.G., Satheesan, K.V. and M.R. Sethuraj. 1986a. Differential photosynthetic response of sun and shade leaves of Hevea brasiliensis to varying light intensities. Proc. TREEPHYSINDIA, 82: in press.

Rao, G.G., Raghavendra, A.S. and Sethuraj, M.R. 1986b. Measurement of water potential in rubber - Comparison of pressure chamber and thermocouple psychrometer-Problems and Perspectives. Int. Rubb. Res. Dev. Board Specialists Group Meeting on Physiology, December, 1986, Hainan, China.

Ribaillier, D. 1970. Importance des lutoides dans l'ecoulement du latex: Action de la stimulation. Rev. Gen. Caout. Plast., 47: 305-310.

Ribaillier, D., Jacob, J.L. and d'Auzac, J. 1971. Sur certains characteres vacuolaires des lutoides du latex d'Hevea brasiliensis Muell. Arg. Physiol. Veg., 9: 423-437.

Riches, J.P. and Gooding, E.G.B. 1952. Studies in the physiology of latex. I. Latex flow on tapping-theoretical considerations. New Phytol., 51: 1-10.

Samsuddin, Z. and Impens, I. 1978. Comparative net photosynthesis of four Hevea brasiliensis clonal seedlings Expl. Agr., 14: 337-340.

Samsuddin, Z. and Impens, I. 1979. Photosynthetic rates and diffusion resistances of seven Hevea brasiliensis Muell.-Arg. clones Biol. Plant., 21: 154-156.

production to tapping. By increasing the biomass production alone a sustained yield increase with age can be ensured. Efforts should be made to elucidate the physiological effects of tapping on yield components. Some more of the unresolved problems in physiology of latex production, warranting further studies are - causes and remedies of Brown bast, mechanism of stimulation by ethylene of the flow and formulation of new chemicals that can enhance flow but do not retard growth/biomass production by trees.

REFERENCES

Abraham, P.D., Boatman, S.G., Blackman, G.E. and Powell, R.G. 1968. Effects of plant growth regulators and other compounds on flow of latex in Hevea brasiliensis. Ann. Appl. Biol., 62: 159-173.

Archer, B.L., Audley, B.G., McSweeney, G.P. and Tan, C.H. 1969. Studies on composition of latex serum and bottom fraction particles. J. Rubb. Res. Inst. Malaya, 21: 560-569.

Archer, B.L. 1980. Polyisoprene. In: Encyclopaedia of Plant Physiology, New series, Vol. 8, Secondary Plant Products, (eds.) Bell, E.A. and Charlwood, B.V., Springer Verlag, Berlin, pp. 309-328.

Bealing, F.J. 1969. Carbohydrate metabolism in Hevea latex - Availability and utilisation of substrates. J. Rubb. Res. Inst. Malaya, 21: 445-455.

Bealing, F.J. and Chua, S.E. 1972. Output, composition and metabolic activity of Hevea latex in relation to tapping intensity and the onset of brown bast. J. Rubb. Res. Inst. Malaya, 23: 204-231.

Benedict, C.R. 1984. Biosynthesis of rubber. In: Biosynthesis of isoprenoid compounds. Vol. II. (eds.) Porter, J.W. and Spurgeon, S.L., John Wiley and Sons, New York, pp. 357-369.

Benedict, C.R., Reibach, P.H., Madhavan, S., Stipanovic, R.V., Keithly, J.H. and Yokoyama, H. 1983. The effect of 2-(3',4-dichlorophenoxy)-triethylamine on synthesis of cis - polyisoprene in guayule plants (Parthenium argentatum Gray). Plant Physiol., 72: 897-899.

Blackman, G.E. 1965. Factors affecting the production of latex. In: Proceedings of the Natural Rubber Producers Research Association Jubilee Conference, Cambridge, 1964. (ed.) Mullins, L., Maclaren, London, pp. 43-51.

Boatman, S.G. 1966. Preliminary physiological studies on the promotion of latex flow by plant growth substances. J. Rubber Res. Inst. Malaya, 19: 243-258.

Boatman, S.G. 1970. Physiological aspects of the exploitation of rubber trees. In: Physiology of Tree Crops, (eds.) Luckwill, L. C. and Cutting, C.V., Academic Press, New York, pp. 323-333.

Buttery, B.R. and Boatman, S.G. 1964. Turgor pressure in the phloem. Measurements on Hevea latex. Science, 145: 285.

Buttery, B.R. and Boatman, S.G. 1966. Manometric measurement of turgor pressures in laticiferous phloem tissues. J. Exp. Bot., 17: 283-296.

Buttery, B.R. and Boatman, S.G. 1967. Effects of tapping, wounding and growth regulators on turgor pressure in Hevea brasiliensis. J. Exp.

Bot., 18: 644-659.
Buttery, B.R. and Boatman S.G. 1976. Water deficits and flow of latex. In: Water deficits and plant growth, Vol. IV. (ed.), Kozlowski, T.T., Academic Press, New York, pp. 233-288.
Camacho, E.V. and Jimenez, E.S. 1963. Resultados preliminares de una proba de induction de floracion prematura en arboles jovens. Turrialba, 13: 186-188.
Ceulemans, R., Gabriels, R., Impens, I., Yoon, P.K., Leong, W., Ng, A.P. 1984. Comparative study of photosynthesis in several Hevea brasiliensis clones and Hevea species under tropical field conditions. Trop. Agric., 61: 273-275.
Chua, S.E. 1966. Physiological changes in Hevea brasiliensis tapping panels during the induction of dryness by interruption of phloem transport. I. Changes in latex. J. Rubber Res. Inst. Malaya, 19: 277-281.
Chua, S.E. 1967. Physiological changes in Hevea trees under intensive tapping. J. Rubber Res. Inst. Malaya, 20: 100-105.
Chua, S.E. 1970. The physiology of foliar senescence ans abscission in Hevea brasiliensis Muell. Arg. Ph.D. Thesis, Singapore Univ.
Chua, S.E. 1976. Role of growth promoter and growth inhibitor in foliar senescence and abscission of Hevea brasiliensis Muell. Arg. J. Rubb. Res. Inst. Malaysia, 24: 202-214.
D'Auzac, J. and Jacob, J.L. 1984. Physiology of the laticiferous system in Hevea. Basis and application to productivity. Proceedings of Intl. Rubb. Res. Dev. Board Coll. Expl. Physiol. Am. Hevea, Montpellier, 1984, pp. 63-79.
de Jonge, P. 1969. Influence of depth of tapping cut on growth and yield. J. Rubber Res. Inst. Malaya, 21: 348-352.
Dickenson, P.B. 1969. Electron microscopical studies of latex vessels of Hevea brasiliensis. J. Rubber Res. Inst. Malaya, 21: 543-558.
Dickenson, P.B. 1976. The application of growth modifier to the production of latex. Outlook Agric., 9: 88-94.
Dijkman, M.L. 1951. Hevea: Thirty Years of Research in the Far East. Univ. of Miami Press, Coral Cables, Florida.
Downton, W.J.S. 1981. Water relations of laticifers in Nerium oleander. Aust. J. Plant Physiol. 8: 329-334.
Evers, E., Verbeke, R., and Marteus, C. 1960. Relations entre le climat, la phenologie et la production de l'Hevea. INEAC, Ser. Sci. No. 84, pp. 1-71.
Frey-Wyssling A. 1952. Latex flow. In: Deformation and Flow in Biological Systems. (ed.) Frey-Wyssling, A., North-Holland Publ., Amsterdam, pp. 322-349.
George, M.J., Satheesan, K.V. and Sethuraj, M.R. 1982. Effect of exploitation methods on biomass production, harvest index and yield in Hevea brasiliensis. Proceedings of PLACROSYM V, in press.
Gomez, J.B. 1982. Anatomy of Hevea and its influence on latex production. Monograph No. 7. Malaysian Rubb. Res. Dev. Board, Kuala Lumpur.
Gomez, J.B. 1983. Physiology of latex (rubber) production. Monograph No. 8, Malaysian Rubb. Res. Dev. Board, Kuala Lumpur, 117 pp.

be also detected by the fall in water potential in the afternoon relative to pre-dawn values (Sethuraj, 1985; Rao et al., 1986b).

BROWN BAST

One of the major maladies affecting rubber trees is "Brown bast", a disorder considered to be a physiological reaction (Rands, 1921). The bark instead of yielding latex, when cut, goes dry and often becomes dark (brown) coloured and hence the name. The dry non-yielding area on the bark may be insignificant or large enough to stop latex yield. Some clones are characteristically more susceptible to brown bast. The only way to overcome this problem is to reduce considerably the intensity or to suspend the exploitation (Chua, 1967), which suggests that the disorder is an over-reaction of the tree to the exploitation.

The reasons for the onset and the ways to overcome brown bast is one of the greatest challenges to the tree physiologists working on rubber. Dryness could be induced on a bark island, isolated by grooves down to the wood, and tapped intensively. In such of these experiments none of the following factors could be confirmed as the reasons of dryness: carbohydrate depletion (Chua, 1966); loss of protein and RNA (Chua, 1967); low osmotic pressure of latex vessels (Boatman, 1970); or the reduction in permeability of latex vessels (Bealing and Chua, 1972).

SCOPE FOR FUTURE WORK

Large variation exists in the latex yields from different trees, which ranges from 6 to 40 l $tree^{-1}$ y^{-1}, and most of the variation is due to genetic differences (Ong, 1978). Although the major yield components are identified (Sethuraj, 1981), several of the subcomponents are yet to be characterised (Sethuraj, 1984). A biometrical approach taking into account the yield components, canopy architecture, assimilatory capacities and growth potentials, can lead to the formulation of an ideo-tree type. Simmonds (1982) recently discussed some of these prospects.

Since rubber trees require 7-10 years to demonstrate their yield potential, it is necessary to evolve methods for early detection of yield potential. New approaches have recently been suggested from China (Zhongyu et al., 1983). Search should be directed to identify such of those physiological and biochemical parameters (e.g. rubber content, growth vigour) which do not show much variation between young and mature plants (Sethuraj, 1985). Efforts should also be made to achieve more vigorous growth of the tree so as to reduce the immaturity period, e.g. polybag plants (Sethuraj, 1985).

Many countries, including India, are venturing to grow rubber in even non-traditional areas, since the land is becoming scarce. These areas usually pose stress conditions such as prolonged drought, low temperature in winter, high altitude and so on. Since clonal variation to stress condition, is a common phenomenon, research should be directed to examine the process of tree adaptation to stress, evaluate the clones and identify the ones suitable for a particular region.

The modelling of latex flow from Hevea is a challenge, acceptable for interdisciplinary research. None of the available mathematical treatments can account for the rates of latex flow in the initial two minutes. With the advent of computer simulation techniques, it should now be possible to solve this problem.

Further detailed studies on the latex vessel plugging may not only reveal the mechanism but also lead to new yield stimulant chemicals. The hypothesis that two distinct types of plugging operate in Hevea (Southorn, 1969) deserves further attention. Similarly the reasons for lutoid damage during flow are yet to be found. Dilution of latex occurs but not to the extent to justify the observed bursting. The possible biophysical/electrical effects (Southorn and Yip, 1968; Lim et al., 1969) may be investigated.

The increase in rubber/latex yields from Hevea, have so far, been through the prolongation of latex flow. No attempt is made to enhance the capacities of rubber biosynthesis in the tree. The recent success in stimulation of rubber hydrocarbon production in guayule (Parthenium argentatum) raises the hope that it may be possible in Hevea too. In guayule, several enzymes of polyprene synthesis were stimulated by the exogenous application of 2-(3,4-dichlorophenoxy)-triethyl amine, DCPTA (Benedict et al., 1983).

Another area that deserves attention is the effect of tapping on growth, since substantial clonal variation exists in the sensitivity of biomass can be partitioned into shoot and root biomass (W_g) and annual rubber yield (Y).

$$W_a = W_g + 2.5\ Y$$

The factor 'k' which indicates the proportion (1-k) of the reduction in biomass potential in a tapped tree even after accounting for the rubber yield, merits attention because by reducing this proportion, W_a can be increased.

$$k = 1 - \frac{W_a}{W_m}\text{, or}$$

$$W_a = W_m\ (1-k)$$

The relationship among the annual biomass increment in an untapped tree (W_m) and the net biomass increment of a tapped tree (W_g) in relation to the amount of rubber extracted out during tapping (Y) and factor 'k' can

TABLE 5

Effect of different exploitation systems on biomass productivity, rubber yield, harvest index and the extent of reduction in biomass production due to tapping (factor 'k') in Hevea brasiliensis (Adapted from George et al., 1982).

Exploitation system	Dry matter increment ($kg\ y^{-1}$)	Dry rubber yield ($kg\ tree^{-1}\ y^{-1}$)	Harvest index	Factor 'k'
		1979-1980		
No tapping	128.5	-	-	-
1/4 S d/2	80.7	7.4	0.19	0.37
1/4 S d/2 + ET*	80.6	8.5	0.21	0.37
1/2 S d/2	79.9	8.0	0.20	0.38
S d/2	2.5	5.6	0.85	0.98
		1980-1981		
No tapping	135.8	-	-	-
1/4 S d/2	59.9	7.4	0.24	0.56
1/4 S d/2 + ET*	64.7	8.4	0.25	0.52
1/2 S d/2	34.0	7.6	0.36	0.75
S d/2	8.9	5.9	0.62	0.93

The notation of tapping system is described in the text.

*ET: Stimulation by ethephon.

be expressed by the following formula:

$$W_g = (1-k) - 2.5\ Y$$

The above concepts are illustrated in Table 5. The factor 'k' affected remarkably the loss in biomass due to tapping as well as the harvest index during a study on growth and biomass production with different exploitation systems in clone RRII 105 (George et al., 1982). The data in Table 5 present the observations for two consecutive years, although the actual experiment was continued for five years.

EFFECTS OF WATER STRESS

The latex, being predominantly watery, its flow from tree, presents one of the classical phenomena influenced by plant water relations (Buttery and Boatman, 1976). Clones of Hevea vary in their sensitivity to water stress (Saraswathyamma and Sethuraj, 1975). But the latex yields are generally reduced at low soil moisture levels, prevalent typically in summer months. The pattern of latex flow was remarkably altered by the soil moisture. The duration of flow as well as the amount of latex were reduced during water stress conditions (Sethuraj and Raghavendra, 1984). The extent of such reduction was marked in clone Tjir 1, while RRII 105 or Gl 1 were influenced only slightly by the soil moisture stress (Fig. 3). In the latter clones, the rate of flow was enhanced during initial stages and subsequently reduced, under stress (Raghavendra et al., 1984).

The drop in latex yields under soil moisture stress was because of enhanced plugging and restricted drainage area (Sethuraj and George, 1976). Premakumari et al. (1980) reported that neutral lipid content of rubber particles and phospholipids of lutoid particles decreased during drought periods. The lipid composition of lutoid membranes changes remarkably under water stress making them fairly unstable leading to an increase in plugging particularly in a drought sensitive clone like Tjir 1 (Raghavendra et al., unpublished).

The drought tolerant clones have been shown to maintain high solute potential in their C-serum (so as to keep lutoids intact) even in summer months (Satheesan et al., 1982). Such ability for osmotic adjustment can

TABLE 4

Partial list of chemicals, reported to stimulate latex flow from Hevea brasiliensis, when applied externally (over the bark just below the tapping cut). A detailed discussion of this subject can be seen in Abraham et al. (1968) and Dickenson (1976).

OILS/PARAFFINS

- Coconut oil
- Linseed oil
- Paraffin oil
- Petrolatum grease

HERBICIDES PLANT GROWTH REGULATORS

- 2,4 - dichlorophenoxy acetic acid (2,4-D)
- 2,4,5 - trichlorophenoxy acetic acid (2,4,5 - T)
- 1 - naphthyl acetic acid (NAA)
- Indole butyric acid (IBA)
- 4 - amino 3,5,6 - trichloro picolinic acid (Picloram)
- 4 - chlorophenoxy propionic acid (CPA)
- 2,4 - dichloro - 5 - fluoro - phenoxy acetic acid (2,4 - Cl - 5 - F)
- α - naphthoxy acetic acid (α - NOXA)
- β - naphthoxy acetic acid (β - NOXA)

ETHYLENE - RELATED

- Acetylene
- 2 - chloroethyl phosphonic acid (Ethephon)
- Ethad*
- Ethylene
- Ethylene oxide
- 1 - aminocyclopropane - 1 - carboxylic acid (ACC)
- Calcium carbide**

MISCELLANEOUS (INCLUDING FUNGICIDAL/BACTERICIDAL COMPOUNDS)

- Cowdung
- Copper sulphate
- Mixture of iron sulfate and potassium permanganate

* A patented formulation where ethylene is trapped physically and released slowly.

** Soil application - releases acetylene under moist conditions.

tissues (Dickenson, 1976). The stimulant is normally applied on the scraped bark just below the tapping cut, for maximum response.

The enhancement of latex yield after stimulation is through lowering the efficiency of plugging process and prolonged duration of flow. Definite reasons have so far not been established (Milford et al., 1969), although several mechanisms are suggested on the action of ethylene on plugging process: changes in the stability of lutoid membrane (Ribaillier, 1970), increase in pH and resultant changes in metabolic pathways (Tupy, 1980), enlargement of drainage area (Pakianathan et al., 1975; Sethuraj, 1985) and changes in biophysical and rheological properties have been suggested (Yip et al., 1974); The direct involvement of ethylene generation in bark, at least, is certain from the observations that a chemical analogue of ethylene, namely acetylene is equally effective in enhancing latex production (Personal communication, South China Academy of Tropical Crops, China).

Repeated stimulation invariably causes a depression of girth increment. Moreover, the response declines with repeated stimulant application. Higher incidence of dry trees (Brown Bast) also has been reported. These observations indicate the need for judicious application of yield stimulants.

EFFECT OF EXPLOITATION ON GROWTH

The process of tapping and the stimulation of latex yield by chemicals constitute the exploitation of rubber tree. The physiological factors influencing growth during the pre-exploitation phase would continue to exert the same effect on trees under tapping. In addition, tapping affects growth.

The annual biomass produced by a tree subjected to regular tapping (W_a) is substantially low compared to that by an untapped tree (W_m). Simmonds (1982) assumed that the biomass loss in a tapped tree can be accounted for by rubber and other products removed in latex. However, Templeton (1969) observed the loss in biomass in a tapped tree may vary almost seven times between clones for comparable rubber yields. The loss in biomass, when a tree is tapped, was not accounted for even if the high energy value of rubber and other substances (2.5 times that of carbohydrates) lost in latex was considered.

The gross biomass realised in a tree subjected to regular tapping (W_a), upon the clone and season. When flow ceases, the tapper collects the latex and takes for processing. In the next tapping day, the cut is reopened by slicing away 1.5 - 2 mm of bark, thereby removing the coagulum which blocks the cut end of the latex vessels. The whole process is repeated on all tapping days. Thus the bark is progressively consumed down the trunk until the bud-graft union; the process takes about 4-5 years. Then a new half spiral cut is opened in the untapped bark on the opposite side of the trunk and the bark of that 'panel' is also consumed in 5-6 years. By this time, the cambial activity would have renewed the bark of the original tapping 'panel' sufficiently, so that 'panel' of renewed bark can be again opened for tapping for another 5-6 years. The same process is repeated on the opposite 'panel' of renewed bark. After this stage various exploitation techniques are adopted including upward tapping to consume the virgin bark above the basal tapping panels. The highest number of latex vessels are situated close to the cambium. Therefore to obtain maximum yields the tapping should be deep upto 1 mm from the cambium (Fig. 2).

There are different tapping systems, based on the variation in the length of the cut, frequency of tapping or number of cuts. The systems are expressed with specific notations. For instance some of the common notations are:

1/2 S d/2 - one half spiral cut tapped every alternate day;
1/2 S d/3 - one half spiral cut tapped every third day;
1/4 S d/2 - one quarter spiral cut tapped every alternate day;
2 x 1/2 S d/4 - Two half spital cuts, tapped every fourth day;
1/2 S d/2 (S x 2d/4) - Two half spiral cuts, tapping panels changed, every alternate day.

The latex yield was maximum and constant between 8.00 pm and 7.00 am and the yield decreases gradually to a minimum of 70 per cent of the maximum yield at around 1.00 pm (Paardekooper and Sookmark, 1969). The diurnal variation in yield is inversely related to the variation in the saturation deficit of the air (Ninane, 1967). The decrease in yield during the course of the day is the result of a lowering of the turgor pressure of the laticiferous system caused by increased transpirational loss of water (Buttery and Boatman, 1964, 1966).

The yield increases with lengthening of the tapping cut, but not proportionately. In other words, the yield per unit length of tapping cut decreases with increasing length of the cut. There is clonal difference in the rate of yield variation due to differences in the length of the cut (Frey-Wyssling, 1952). The ratio of yield to different lengths of the tapping cut is dependent upon the drainage area, which again is a clonal character; the larger the drainage area, the lesser would be the effect of increasing the length of tapping cut on yield. The other factor associated with length of tapping cut is plugging index. Lengthening of the tapping cut tends to lower the plugging index, resulting in enhanced yield (Southorn and Gomez, 1970). Hence a clone with low plugging index will have less response to variations in the length of tapping cut. However, lengthening of the cut to full spiral has a depressing effect on girth increment (De Jonge, 1969; Ng et al., 1970).

Single cut tapping systems are suitable for young trees as double cut systems adversely affect girthing. The results of many experiments indicate that double cut systems would be profitable after the fourth panel (Ng et al., 1970).

Frequency of tapping is a factor which would influence the physiology of the tree as well as the yield from the tree. While a high frequency of tapping would affect the physiological balance between extraction of latex and its replenishment in the latex vessels, too low a frequency would result in reduced yield. Increasing the frequency tends to increase the incidence of brown bast syndrome.

YIELD STIMULATION

The volume of latex on tapping can be increased by application of chemicals on the bark. Such yield stimulation was achieved by a wide range of compounds like 2,4-D or 2,4,5-T (synthetic auxins), mineral oil or copper sulphate (Table 4). A comprehensive screening of various chemicals having different physiological functions indicated that chemicals which can generate ethylene are effective yield stimulants (Abraham et al., 1968). This finding led to the wide-spread use of ethephon (2-chloroethyl phosphonic acid) as a stimulant as this chemical disintegrates evolving ethylene in plant (micro-climate influenced by canopy architecture, leaf area index and meteorological inputs; density of planting).

Some of the assimilates produced in an untapped tree are utilised for rubber biosynthesis in the laticiferous system. When a tree is tapped, a part of latex thus synthesised is extracted. It is believed that the loss of latex by tapping triggers its re-synthesis. The ratio of rubber yield per tree per year (Y), to the total biomass per tree per year (inclusive of rubber extracted) (Wa), is defined as harvest index (c). In calculating the harvest index, only the rubber extracted is considered and the high calorific value of rubber (2.5 times that of carbohydrate) is accounted:

$$c = \frac{2.5\,Y}{Wa}$$

A peculiar situation in Hevea is that the yield of latex from a tree is determined by not only the inherent factors and the environment but also by the exploitation methods adopted. The sink volume can be altered, by changing the exploitation systems. The value of 'c' is thus regulated by the quantity of the rubber (Y) extracted, unlike in other crops where basically the inherent factors influencing 'c' determine the yield.

The yield of rubber from a tree on each tapping is determined by the volume of latex and percentage of rubber it contains. Sethuraj (1981) derived a formula, ascribing the variation in the production of rubber by any tree through four major yield components:

$$y = \frac{F.l.C_r}{p}$$

where y is yield of rubber obtained from a tree each time it is tapped; F - the average initial flow rate per cm of taping cut during the first 5 min after tapping; l - the length of the cut; C_r - dry rubber content of the latex and p - the plugging index, a measure of the extent of latex vessel plugging.

Each of the above major yield components is influenced by several internal and external factors. The rubber content is determined by the extent of assimilate partitioning into rubber. The length of the cut which is a direct proportion of the girth of the tree, in turn, is dependent on annual biomass increment and it is known that tapping can result in biomass loss which cannot fully be accounted for by rubber yield (see EFFECT OF EXPLOITATION ON GROWTH).

Of the four major components in the formula, the factors influencing l (related to girth of the tree) have already been discussed. All the other three components also are influenced by the exploitation systems and clonal characters. Some of the important sub-components of these major components are listed below. This list is only indicative and not complete. Many biochemical components such as carbohydrates, enzymes of carbohydrate metabolism and rubber biosynthesis (invertase to polymerase) may exert their influence on one of the major components directly or through the sub-components listed.

Sub-components of major components

Initial flow rate (F):

* Number of latex vessel rings
* Diameter and other anatomical characters of latex vessels
* Turgor pressure at the time of tapping

Plugging index (p):

* Rubber particle stability
* Lutoid particle stability and the composition of lutoid membrane
* Flocculation potential of lutoid serum
* Interaction between C-serum and B-serum
* Dilution reaction on tapping
* Mineral composition of latex
* Drainage area

Rubber content (C_r):

* Rubber biosynthetic capacity
* Level of exploitation

TAPPING

Latex from the tree is obtained by cutting open the latex vessels by a process known as tapping. The most common method of tapping is to cut a half spiral groove in the base, at an angle of 25° - 30° from the left to right. The first cut is made at a height of 125 - 150 cm when the tree attains a girth of 50 cm at that height. This is done with a special knife, and aims at cutting the bark to within 1 mm of the cambium. Latex flows down the cut to a metal spout fixed at the right end and thence into a collection cup (Fig. 1). Duration of latex flow may be from 1-3 h depending

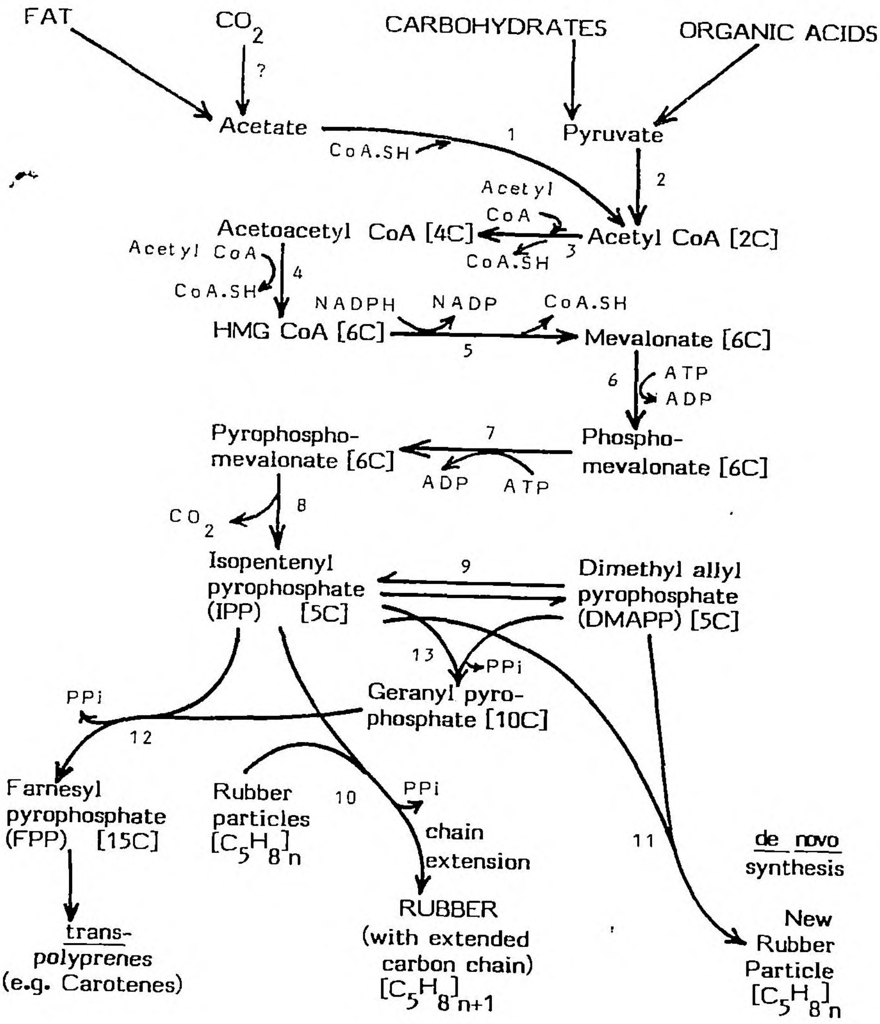

Fig. 5. Biosynthesis of rubber hydrocarbon. The basic unit of isoprene (IPP) leads to the formation of either rubber (cis 1,4- polyisoprene), carotenes or polyterpenes. The numbers indicate the relevant enzymes - 1: Acetate CoA synthetase; 2: Pyruvate dehydrogenase; 3: β-ketothiolase; 4: HMG CoA condensing enzyme; 5: HMG CoA reductase; 6: Mevalonate kinase; 7: Phosphomevalonate kinase; 8: Pyrophosphomevalonate anhydrodecarboxylase; 9: Isoprene isomerase; 10: Rubber transferase; 11: Condensing enzyme; 12: FPP synthase; and 13: Prenyl transferase.

phate (IPP). Although the polymerisation of IPP and its isomer dimethyl allyl pyrophosphate (DMAPP) could result in a new rubber particle, de novo synthesis has seldom been observed. However the extension of the already existing rubber hydrocarbon chain can be easily demonstrated at the inter-phase between serum and rubber particles (Lynen, 1969). A summary of the proposed pathway is given in Fig. 5. The studies on rubber biosynthesis by Hevea were limited by the non-availability of experimental techniques. For e.g. the latex of Hevea, in vitro, could incorporate only small amounts of ^{14}C-acetate or ^{14}C-pyruvate into rubber, presumably because of the absence of mitochondria and other organelles (Lynen, 1969). Of late, considerable progress is made on rubber biosynthesis in guayule (for a recent article, see Macrae et al., 1986).

Latex contains large amount of sucrose (apart from quebrachitol, an inositol) as well as enzymic components of glycolysis, respiration as well as Embden-Meyerhoff-pathway (Bealing, 1969). The rapid decrease in sucrose along with the increase in invertase activity suggests that the carbohydrates are the main precursors for rubber biosynthesis. Rubber yield is positively related to carbohydrate metabolism in latex as well as the ability to incorporate substrates into rubber hydrocarbon (Recent ref: Low and Gomez, 1984; Tupy, 1985; Leang et al., 1986). Some of the rate limiting steps of rubber biosynthesis are identified as sucrose supply to laticiferous tissue, invertase catalysed hydrolysis of sucrose, HMG-CoA reductase and the ratio of invertase to rubber polymerase (D'Auzac and Jacob, 1984).

PHYSIOLOGY OF LATEX PRODUCTION

Most of the studies aimed at identifying physiological factors influencing yield were confined to analysing the immediate factors determining the yield per tree per tap. Rubber yield from an unit of land area during the economic life span of the tree, however, is influenced by the stand per hectare, biomass production and the extent of partitioning into rubber. Therefore, the present analysis of rubber yield from a plantation, attempts to take into account all these factors. The biomass production in a untapped tree (Wm) is governed by both inherent potential of the tree (relative rates of photosynthesis, partitioning and respiration) and environmental variations suggest that the drainage area, a nearly oval region (in case of half spiral cut), extends from 40 to 120 cm below cut and from about 20 to 100 cm above the cut (Gomez, 1983). The extent of drainage area may vary with clones, chemical stimulation or the environmental stress.

PLUGGING

The gradual restriction of the flow, after tapping, because a physical plug forms near the cut surface of latex vessels (Boatman, 1966; Southorn, 1969). The rapidity of 'plugging' (resulting in a decline in the rate and ultimately the cessation of flow), along with the initial rate of flow, determines the total latex yield from any given tree. These observations led to the formulation of 'plugging index' (Milford et al., 1969), defined which corresponds to the time-flow constant 'a' in the die-away equation ($y = be^{-at}$) discussed in earlier pages.

Plugging index 'a' can be estimated as follows:

$$a = \frac{\text{Mean flow rate, within the first 5 min (ml min}^{-1}\text{) x 100}}{\text{Total latex yield (ml)}}$$

The plugging index is a clonal characteristic (Paardekooper and Samosorn, 1969) but is affected by the exploitation techniques as well as the environmental stress. However the variation in the latex yield can invariably be accounted to a great extent to the corresponding changes in plugging index (Sethuraj, 1981). Modifications of plugging index have been suggested (Sethuraj et al., 1978; Yeang and Daud, 1984) but the above formula of Milford et al. (1969) remains the most popular one.

The latex vessel closure (plugging) is a 'classical phenomenon of interaction between rubber particles and lutoid vesicles (Southorn and Yip, 1968). The rubber particles try to maintain their stability, while the lutoids, when broken, destabilise the rubber phase leading to the formation of a coagulum. The fluid contents of lutoids or B-serum causes flocculation of rubber particles. The exact agents are not identified but factors like acid pH, high levels of divalent cations like Mg^{2+} or cationic proteins, might be the reason for B-serum activity (Southorn, 1969; Gomez, 1983). The integrity of lutoids, therefore, contributes to latex stability. On the other hand, the C-serum of latex, by providing an isotonic osmoticum as well as a suitable chemical microenvironment (high monovalent ions, basic proteins, neutral or basic pH), keeps lutoids intact. Thus counteracting against B-serum, C-serum promotes dispersion of rubber phase (Southorn and Edwin, 1968). The total latex yield is inversely related to plugging index while a positive correlation is established between plugging and bursting indices (Southorn, 1969). The triglyceride and phospholipid contents of latex reflect the stabilities of rubber particles and lutoids, respectively (Ho et al., 1975; Sheriff and Sethuraj, 1978) whereas the activities of B- and C- sera promote the flocculation and dispersion of rubber particles (Southorn and Yip, 1968).

Latex vessels are plugged with flocs of rubber particles and broken lutoids, initiated by the release of B-serum into the latex. Though the increase in the population of damaged lutoids is evident during latex flow (Pakianathan et al., 1966), the main reason for lutoid damage is not established. It may be due to the osmotic (dilution), mechanical (shearing), electrical (wound induced potential) or chemical (bark factors) effects encountered during tapping (Gomez, 1983; Yip and Gomez, 1984).

The extent of lutoid damage can be estimated by the observations from ultracentrifuge pattern, light or electron microscopy or more precisely by the "bursting index" determined as follows:

$$\text{Bursting index} = \frac{\text{Free acid phosphatase activity} \times 100}{\text{Total acid phosphatase}}$$

Acid phosphatase occurs only in lutoids. The level of the enzyme in the serum represents the extent of release from damaged lutoids. The total activity is assayed by the disruption of lutoids with detergents like Triton X Ribaillier, 1970).

The amount of latex that flows out from the cut increases steadily with successive tappings until it reaches a steady state while the dry rubber content decreases. However the extent of increase in the volume of latex is much more than that of the decrease in rubber content and thus the rubber yield $tree^{-1}$ tap^{-1} improves considerably.

Biosynthesis

The biosynthesis of rubber hydrocarbon has been dealt extensively in two recent reviews (Archer, 1980; Benedict, 1984). Being a (cis 1,4-) polyisoprene, the basic unit for rubber biosynthesis is isopentenyl pyrophos-

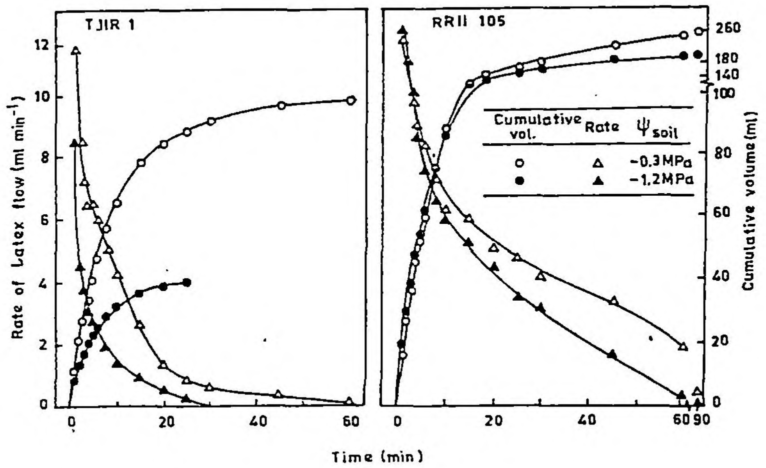

Fig. 3. Kinetics of latex flow at two levels of soil water potential in a fast plugging clone Tjir 1 and slow plugging clone RRII 105. The rate of flow and cumulative volume are presented.

very fast. During second phase latex flow settled down to a slow rate which continued until it finally stopped.

Several investigators attempted to derive a model for latex flow (Frey-Wyssling, 1952; Riches and Gooding, 1952; Paardekooper and Samosorn, 1969; Gomez, 1983). The best among them is the die-away expression ($y = be^{-at}$, in which the flow rate 'y' at a given time 't' is a function of the initial flow rate 'b' and a time-flow constant 'a') derived by Paardekooper and Samosorn (1969). However the initial rates during the first 1 or 2 min after tapping always deviated away from any of these models. Recently Raghavendra et al. (1984) proposed that these two phases of latex flow should be treated separately and that the first phase, upto four min fitted into curvilinear equation while the second phase was an exponential curve.

The turgor pressure in laticifers is suddenly released due to opening of the vessels. In a typical experiment with RRII 105 the high turgor pressure of 1.2 MPa in latex vessels, fell steeply to a low 0.2 MPa by 5 min and increased again to about 0.7 MPa by 30-45 min (Fig. 4). The quick increase ,

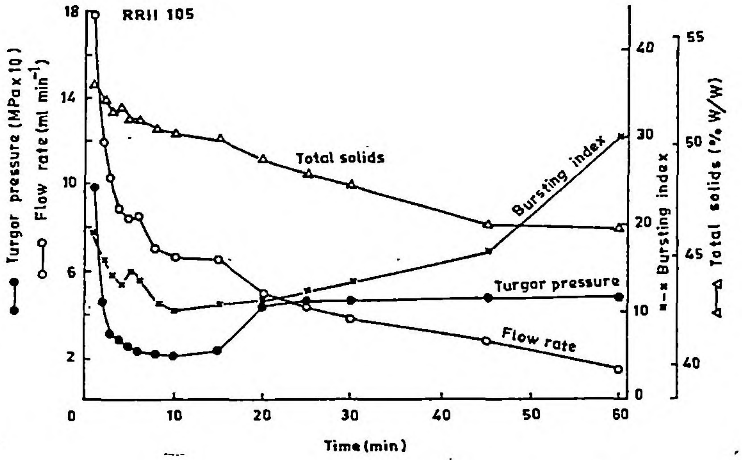

Fig. 4. The course of turgor pressure in latex vessels, bursting index of lutoids in relation to the rate of flow and dilution as indicated by dry rubber content in RRII 105.

within 10 min, suggests the internal plugging of vessels. A steady dilution of latex occurs during the flow as indicated by the decrease in not only the osmotic potential but also the total solids as well as the dry rubber content (Buttery and Boatman, 1976; Gomez, 1983).

The hypothetical area of bark on the stem from which the latex flows out on tapping, is designated as "drainage area" (Frey-Wyssling, 1952). The structure and extent of drainage area is of great importance in latex production by any given tree since it is not only the site of storage but also the biosynthesis of latex/rubber. Several methods have been employed to estimate drainage area in Hevea - rubber or total solid content of latex (Gooding, 1952); stem contraction due to tapping (Lustinec et al., 1969); introduction of radioisotopes into latex (Lustinec and Resing, 1965); profiles of turgor pressure in latex vessels (Pakianathan et al., 1976); yield of micro-tappings (Lustinec and Resing, 1965); or tapping induced changes in mineral or enzymic content of latex (Lustinec et al., 1966). Several of these studies

(Fig. 2). But care need be taken not to injure the cambium (so that bark regeneration is not affected).

The latex collected by tapping constitute the modified cytoplasm from the inner region of laticifers. The adjacent sieve tubes and other cells near the cut do not seem to make much contribution (Gooding, 1952; Riches and Gooding, 1952). Spherical or pear-shaped rubber particles, which range in diameter from 100 A° to 5 µm occupy a major proportion of latex. The other non-rubber particles of latex include lutoids (fragile vesicles limited by an unit membrane, with a diameter of 2-10 µm and are often equated to vacuoles) and Frey-Wyssling complex of 4-6 µm in diameter rich in lipids and carotenoids (Dickenson, 1969). These occur in proportions of 25-45% rubber, 10-20% lutoids and 1-3% Frey-Wyssling complex (Southorn, 1969). lipids

A strong protective lipoprotein layer exists around the rubber particles. This acts as a negatively charged envelope of hydrophilic colloids. Lutoids also are bounded by a thin but complex membrane consisting of proteins and lipids (particularly sterols and phospholipids). The inner content of lutoids (which can be collected by freezing and thawing), referred to as B-serum contains high levels of hydrolytic enzymes such as acid phosphatase, cations like Mg, Ca or Cu and organic compounds like citrate and phosphate (Pujarniscle, 1970; Ribaillier et al., 1971).

These particles are suspended in a soluble phase called C-serum, constituting an isotonic osmotic medium for keeping the organelles intact. C-serum is shown to possess large amount of carbohydrates (particularly quebrachitol and sucrose), proteins and ions like K^+ (Archer et al., 1969).

A much detailed description of the cytology and ultra-structure of latex/laticifers is given in two monographs (Gomez and Moir, 1979; Gomez, 1982).

MECHANISM OF FLOW

The latex flows out when a tapping cut is made on the trunk of the tree, mainly because of the very high turgor pressure in the latex vessels (Buttery and Boatman, 1964; Raghavendra et al., 1984). Turgor pressures in laticifers of Hevea, which reach upto 1.5 MPa are among the highest recorded in laticifers of different species (Table 3). The turgor of latex

TABLE 3

Turgor pressure in laticifers of different plant species.

Species	Observed pressure range (MPa)	Reference
Hevea brasiliensis	0.8 - 1.5	Buttery and Boatman, 1966
	0.2 - 1.2	Milford et al., 1969
	0.8 - 1.2	Raghavendra et al., 1984
Crypostegia grandiflora	1.0 - 1.2	Raghavendra, unpublished
Ficus elastica	0.8 - 1.0	Buttery and Boatman, 1966
Euphorbia pulcherrima	0.7 - 0.8	Buttery and Boatman, 1966
Bursera microphylla	0.7	Downton, 1981
Nerium oleander	0.1 - 0.6	Downton, 1981

vessels is maximum during dawn, falls during day and gets rebuilt in the night (Buttery and Boatman, 1967). The poor yield of latex when the trees are tapped much after sunrise is believed to be due to such diurnal variation in the turgor of latex, which in turn could be due to the changes in water vapour deficit in the air (Paardekooper and Sookmark, 1969). A gradient of turgor pressure with height has been recorded in Hevea stem, with greater pressure in the base than that in the upper region. A gradient upto 0.6 MPa/ 10 m existed during day and decreased to 0.1 MPa/10 m in the night (Buttery and Boatman, 1966).

The initial flow of latex is due to the elastic contraction of walls when the fluid cytoplasm of latex vessels is expelled after a sudden release in their turgor (Southorn, 1969; Boatman, 1970; Buttery and Boatman, 1976; Gomez, 1983). After a while, the flow is regulated by capillary forces until the flow ceases as the latex coagulates and plugs the vessels (Boatman, 1966; Milford et al., 1969). The existence of two distinct phases during the course of latex flow has been demonstrated in several clones (Raghavendra et al., 1984). The duration of flow was less and rates of initial flow more in clones like Tjir 1 than those in ones like RRII 105 (Fig. 3). In the first few minutes, the expulsion of latex was rapid and the rate decreased

Further studies on the pattern of canopy architecture in relation to light interception may help to evolve an ideal tree type.

Biomass production and assimilate partitioning

The biomass production by rubber tree, reaching upto 35 to 50 t ha^{-1} y^{-1} (Templeton, 1968; George et al., 1982), is among the highest recorded from crop species. However the process of exploitation and extraction of latex, limit dry matter production to the extent of 10 - 60% since rubber production involves partitioning of assimilates and too much draining of metabolic energy. As per Templeton's estimate (1969), rubber harvested during first two years accounted for 3 to 11 per cent of total dry weight but this proportion was expected to increase upto 20% or more in subsequent years. The percentage depression due to tapping of trunk growth which is much greater than that of canopy, causes significant loss in rubber yield, since the girth is one of the components determining yield. Such reduction is because of not only the loss of vital metabolities through latex serum, but also the high energy requirement for the resynthesis of rubber (polyisoprene with a calorific content 2.5 times as that of glucose).

Rubber trees are tapped when the girth of their stem reaches a stipulated minimum (50 cm) and the trees below this level are considered 'immature'. It is therefore desirable to promote early growth of young trees so as to reduce the immaturity period (Sethuraj, 1985).

The use of elite planting material and improved agro-management techniques could help in achieving quicker growth during immaturity period (Sivanadyan et al., 1975; Sethuraj, 1985). However, the difference in the growth rate and girth increment between vigorous and non-vigorous plants slowly disappears with the onset of branching and spread of canopy (Templeton, 1968). The importance of mutual shading is also reflected in the absence of any significant effect of plant density on the growth rate of a plant, until the canopy is closed (Dijkman, 1951; Templeton, 1968). During the studies at Malaysia over a seven year period, Templeton (1968) observed that the rate of dry matter production reached the maximum at 5 years after budding and decreased over the next two years.

A steady decline in the relative growth rate (RGR) upto thirtynine months was the result of progressive fall in the net assimilation rate (NAR).

Subsequently both the NAR and leaf area ratio (LAR)-declined; the latter due to the increasing proportion of non-photosynthesising plant tissues. The decline in NAR on the other hand was mainly due to the increase in the shading of leaves within the canopy because of the maximum leaf area index (LAI) was already reached. The maximum values of LAI (5.8 to 6.3), obtained after about 5 years from planting (Shorrocks, 1965; Templeton, 1967) corresponded with the period of maximum dry matter production (24 to 35 t ha^{-1}) and resulted in about 2.8% efficiency of solar energy utilisation (Templeton, 1969).

ANATOMY OF LATICIFERS AND LATEX COMPOSITION

Latex is located in the concentric rings of laticiferous vessels concentrated in the zone of secondary phloem (Gomez, 1982). Each ring is constituted by articulated anastomosing latex vessels which run longitudinally. During commercial extraction of latex (see TAPPING), the cut is made in such way so as to cut through a maximum number of latex vessel rings

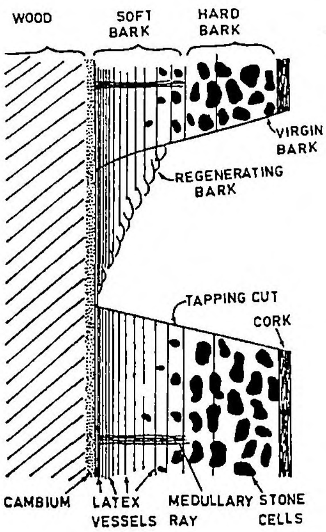

Fig. 2. A longitudinal section of the stem of rubber tree representing the location of laticiferous tissue. The rings of latex vessels, situated in the phloem, are concentrated towards cambium. The tapping cut should reach through the phloem as near to the cambium as possible (yet not touching it) for maximum latex yield. See also Fig. 1.

shed their leaves almost completely before the new growth flushes begin. Considering the season of leaf fall this phenomenon can be more correctly characterised as summer defoliation (Chua, 1970). The degree of flushing depends on the environmental, clonal or individual variation in wintering. However, the clonal differences in wintering becomes more evident in regions of pronounced dry season (Evers et al., 1960). The exact mechanism for periodical abscission of leaves is not yet understood but hormonal control of protein synthesis and general metabolic activity might be involved (Chua, 1976). Very few studies are made on flowering in Hevea (Evers et al., 1960; Camacho and Jiminez, 1963). Flowering, which normally occurs once an year, is initiated after leaf fall.

The density of planting affects the canopy architecture and may affect the growth rate of mature trees. The crotch height of trees increases under higher densities of stand while the girth of individual tree decreases (Westgarth and Buttery, 1965; Satheesan et al., 1986). The stand per hectare does not affect the LAI as the trees adapt by changing the branching habit. However the total biomass per unit land area increases with density (Table 1).

Photosynthesis

The photosynthetic characteristics of individual leaves of rubber tree have been studied (Samsuddin and Impens, 1978, 1979; Ceulemans et

TABLE 1

Canopy characters and dry matter production in twelve year old Hevea (Clone RRII 105) under two planting densities (adapted from Satheesan et al., 1986).

Character	Planting density 4.26 x 4.26 m	Planting density 5.7 x 5.7 m
Leaf area index	3.77	3.78
Spread of canopy (cm)	234.4	377.4
Crotch height (cm)	640.1	340.9
Girth (cm)	77.5	90.5
Dry weight (kg $tree^{-1}$)	486.9	732.1
Dry weight (t ha^{-1})	263.1	215.4

al., 1984; Rao et al., 1986a; Satheesan et al., 1984) but comprehensive estimates of canopy photosynthesis are not available. The carbon assimilation pattern of the leaves have not been reported. The lower range of maximal photosynthesis rates and CO_2 compensation points suggest rubber tree to be a C_3 plant (Samsuddin and Impens, 1978, 1979; Rao et al., 1986a). The net photosynthesis and gas exchange characteristics of leaves differ with clones. However, the validity of photosynthesis measurements as a tool in plant selection from nursery is questioned (Samsuddin et al., 1985).

The profile of photosynthetically active radiation (PAR) reaching the canopy of rubber tree, exhibits a declining gradient towards the lower strata of the canopy (Table 2). Although the shade leaves are adapted to lower light regimes, the maximum photosynthetic rate of shade leaves is low compared to sun leaves (Satheesan et al., 1984; Rao et al., 1986a).

TABLE 2

Photosynthetically active radiation (PAR) budget of three strata in Hevea canopy (adapted from Satheesan et al., 1984).

Observation	Clear day			Cloudy day		
	Top stratum	Middle stratum	Bottom stratum	Top stratum	Middle stratum	Bottom stratum
Incoming photosynthetic photon flux density (%)						
Reflection	2.3	0.5	0.4	2.8	0.6	0.4
Transmission	10.3	7.2	6.2	12.4	9.2	5.6
Interception	87.4	92.3	93.4	84.8	90.2	94.0
Average photosynthetic photon flux density ($\mu E\ m^{-2}\ s^{-1}$)		1083			689	
Day's integration ($E\ m^{-2}$)		49			31	

Height of strata - Top: 9.9 m from the ground level; Middle: 6.7 m from the ground level; Bottom: 2.7 m from the ground level.

Fig. 1. Tapping of a rubber tree. The white milky latex flows along the edge of the cut, and (guided by the metal or plastic spout) collects into a container (a coconut shell is used in this instance). The location of latex vessels is shown in Fig. 2.

The physiology of latex production has been periodically reviewed by Blackman (1965), Sethuraj (1968, 1985), Southorn (1969), Boatman (1970), Buttery and Boatman (1976), Moraes (1977), Gomez (1983) and has been the topic of a recent colloquium (IRRDB, 1984). Readers interested in the general aspects of the rubber tree are referred to the monographs of Dijkman (1951) and Rubber Research Institute of India (Pillay, 1980).

GROWTH AND PRODUCTIVITY

Rubber is propagated either through seeds or vegetatively, by budgrafting. Unselected seeds and seeds from monocrop gardens are discouraged and when seeds are to be used, only polyclonal seeds (clonal seeds collected from polyclonal areas) are recommended. However, most preferred method of propagation is through budgrafting. Seeds start germinating 6 to 8 days after sowing. The mode of germination is hypogeal. The rubber seeds are viable only for a very short period; and therefore, should be sown as early as possible after collection.

Root growth

The tap root of Hevea grows very deep into a normal light soil, reaching more than 10 m down into earth (Moraes, 1977). The lateral roots, in fact, exceed the tap root in length, particularly in young trees, for e.g., lateral roots are 6-9 m while tap root is 1.5 m in 3 year old trees; 9 m long lateral roots on 2.4 m long tap root in 7-8 years old trees. However in shallow alluvial soils the tap roots are confined to the top zone of 'A' horizon but are compensated by profuse growth of laterals. In a plantation, the root density between rows increases steadily until it reaches an uniform level.

The efficiency of root growth in Hevea is illustrated by lower shoot to root ratio, higher root respiration and greater mineral absorption in 3 year old Hevea seedlings than those in cacao of similar age (see Moraes, 1977). Although endotrophic mycorrhiza have been found in roots of Hevea (Waistie, 1965) their assistance in nutrient uptake is not yet demonstrated.

Shoot growth

A remarkable seasonal periodicity exists during shoot growth of Hevea where periods of rapid shoot elongation and leaf expansion are alternated with periods of inactivity (Moraes, 1977). In young unbranched trees, leaves of each flush form a distinct layer on the stem, and each year may produce 2-4 flushes. Adult trees exhibit prominent annual flushing periodicity. Since periods of flushing coincide with those of cambial activity, the number of latex vessel rings in the stem are positively related to the flushing frequency (Halle and Martin, 1968).

Another feature of adult Hevea trees is 'wintering', when the trees

In: Tree crop physiology.
Eds C MR Sethuraj and A S Raghavendra,
Elsevier. Sc Pubns, Amsterdam, pp 193-20