Q. Jl. Rubb. Res. Inst. Sri Lanka (Ceylon) (1973) 50, 19—27

AN ASSAY OF GROWTH INHIBITORS IN SEED OIL AND NORMAL AND DISEASED (BROWN BAST) BARK OF *HEVEA*

BY

C. M. B. RATNAYAKE

INTRODUCTION

Though the yield of a rubber tree is governed by its genetic constitution the extraction of latex has a profound effect on the physiology of the tree; this may in turn affect yields. One of the syndromes produced by over-exploitation is browning and drying of the bark known as 'Brown Bark' or 'Brown Bast'. With the adoption of modern tapping systems and methods of stimulation of yeild, this disease has assumed greater significance. Early studies have demonstrated a close relationship between Brown Bast and tapping intensities and greater susceptibility in higher yielding clones (Bobilioff, 1925 and Frey-Wyssling, 1929). Therefore the *Hevea* breeder is never sure whether, in breeding and selecting for increased yields, he is unwittingly selecting for higher Brown Bast susceptibility.

The onset of Brown Bast is always preceded by the production on tapping, of increased volumes of latex of lower dry rubber content(d.r.c.); subsequent yields decrease gradually to zero value and usually browning of the bark increases. Compagnon, Tixier & Roujansky (1953) have described five classes of Brown Bast according to symptoms (1) browning of the tapping cut due to spots, cracks in the bark and stoppage of latex flow (2) deformation of the panel (3) coagulation of latex on the tapping cut (4) partial dryness without any colour (5) total dryness without any colour change of the bark; occasionally all or many of these characters may appear in the same tree. Rands (1921a) considered this disease to be a local wound phenomenon; Rhodes (1930) related it also to a wound healing phenomenon; Frey-Wyssling (1929) attributed the diseases to the formation of tyloses in the younger wood vessels in the xylem, and Eber(1937) found that these tyloses could be formed when the water content in the xylem drops by about 25%. Vollema (1937) showed that disease incidence was at a maximum soon after wintering. Schweizer (1949) postulated the non-availability of organic food in the bark to be the cause of the disease. Schweizer, Compagnon & Roujansky (1953) have shown that the disease could be induced by tapping several times a day. Chapman (1951) has indicated variations in hormone concentrations in trees of varying yield capacity. Beaufils (1954) observed a high magnesium in the latex of diseased trees and considered mineral metabolism to be the causative factor leading to precoagulation of latex on the tapping cut. More recent work in Malaysia (Anon, 1965) does not demonstrate any relationship between Brown Bast incidence and the nutrient status of the soil. The aetiology of the disease remains very obscure and the excessive extraction of latex appears to be the main predisposing factor. No treatment has yet been recommended except for a resting period of five to six months for affected trees. Scraping of the effected area and the application of stimulants have also been practised. These studies were undertaken to clarify whether the diseased condition is associated with growth substances present in other tissues of *Hevea*.

MATERIALS AND METHODS

Initially a search was made for a local plant material as a substitute for *Avena* (Oat coleoptiles, which are generally used in temperate countries, to test auxins. Other plant materials tested included stem apices of *Mikania, Pueraria phascoloides,* and *Vitis riparia,* (grape) tendrils of *Passiflora edulis* and *Vitis riparia,* and flower stalks ot *Coreopsis.*

Indole acetic acid (IAA) solutions of 0.05%, 0.025%, 0.01%, 0.005%, 0.0025%, and 0.001% concentration were prepared in 2% sucrose solution. A 2% sucrose solution was used as the control. Ten 6 mm pieces from each plant material were treated with each concentration for 24 h. They were measured before and after treatment and compared with the control in 2% sucrose. Measurements were made using the vernier sub-stage of the micorscope at a magnification of 40x.

RESULTS

Selection

As shown in Fig. 1 the average increase in length of the ten pieces of each plant material was plotted against IAA concentration. It is seen from the graph that grape and *passiflora* sections increase gradually from 0.001% to 0.0025% IAA concentrations; increase of concentration above 0.005% induced inhibition and some fluctuation, possibly due to toxicity. *Pueraria* showed sensitivity over a wider spectrum of concentrarion but there was appreciable variability in response on repitition of the experiment. *Mikania* and *Coreopsis* sections showed better reproducibility, and wider range of sensitivity than previously described materials. The tendency for the section to curve due to uneven increase on the two sides was also less in the case of *Mikania* and *Coreopsis.* Stem apices of *Mikania* from one but the last internode were found to show greater sensitivity than *Coreopsis* flower stalk sections.

Growth substances in rubber seed oil

In view of the inverse relationship between oil content and growth in *Hevea* shown by Fernando & de Silva (1971) an attempt was made to isolate growth substances present in the cotyledons and test them for activity.

The shells of the rubber seeds were removed and the cotyledons (and embryo) dried for 12 h. in a vacuum oven. They were then powdered using a high speed macerator and oxtracted with ether using a Goldfish extractor. The ether was evaporated and aliquots of 10, 15 and 25 were spotted on Whatman No. 4 and No. 1 Chromatographic papers. Descending paper chromatographic separation was carried out using Isopropanol: Ammonia: Water (10: 1: 1V/V). After an equilibration period of 14 h. the solvent was run for a distance of 30 cm in complete darkness. The chromatograms were dried at low temperature (below 30°c) for 24 h. in an oven fitted with an air-circulating fan. Ten pieces from Rf. 0.1 to 1.0 were cut from each strip and placed in separate small petri dishes containing 5 cc of 2% sucrose solution; a control piece of filter paper was also placed in 2% sucrose solution. The perti dishes were kept closed overnight and 10 sections each of *Mikania* and *Coreopsis* were placed on them. After a further 24 h. the sections were measured microscopically at a magnification of 40x. The difference in length was calculated as compared to the control.

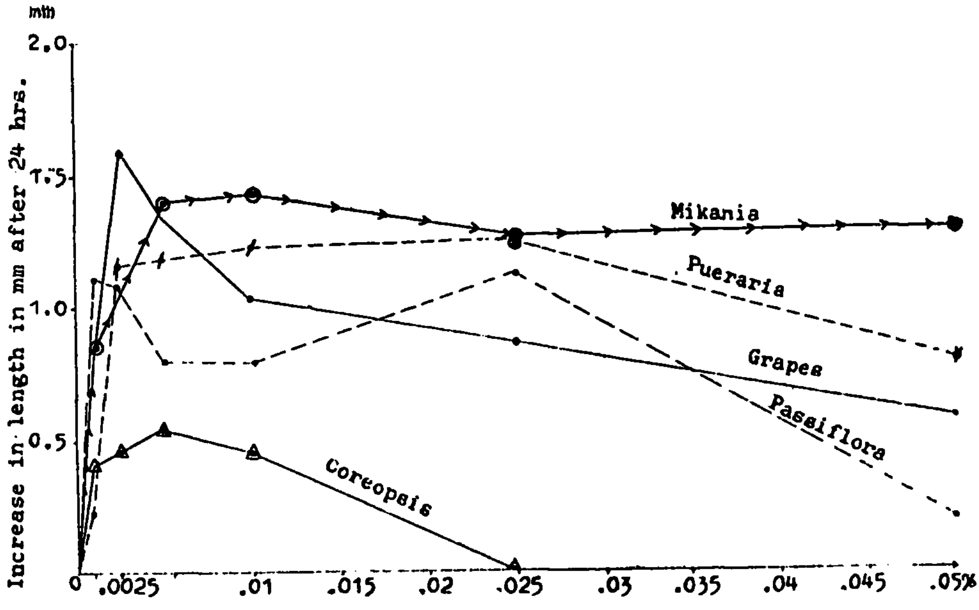

Figure 1. Sensitiveness of five plant materials to various concentrations of I.A.A

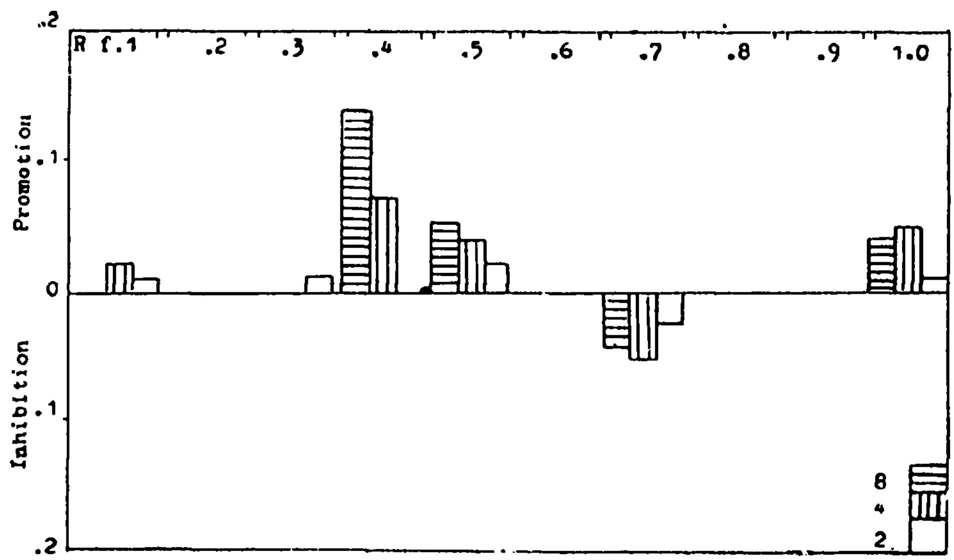

Figure 2. Growth substances present in rubber seed oil. Extracted with ether and tested with *Mikania* at three concentrations (8,4,2) (Only the significant values are included. L.S.D.=0.07 for 8 strips)

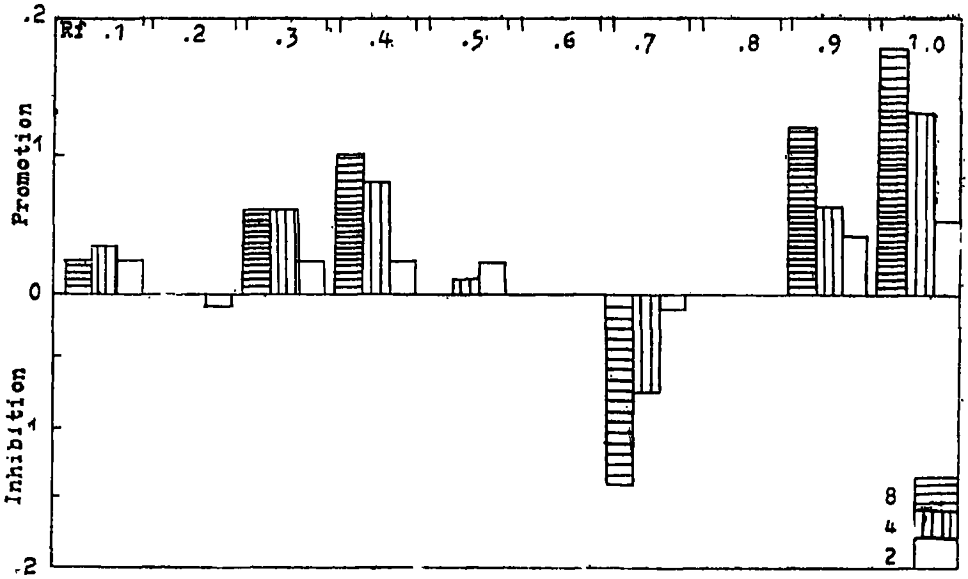

Figure 3. Growth substances present in rubber seed oil. Extracted with ether and tested with *Coreopsis* at three concentrations (8,4,2) (Only the significant values are included. L.S.D.=0.05 for 8 strips)

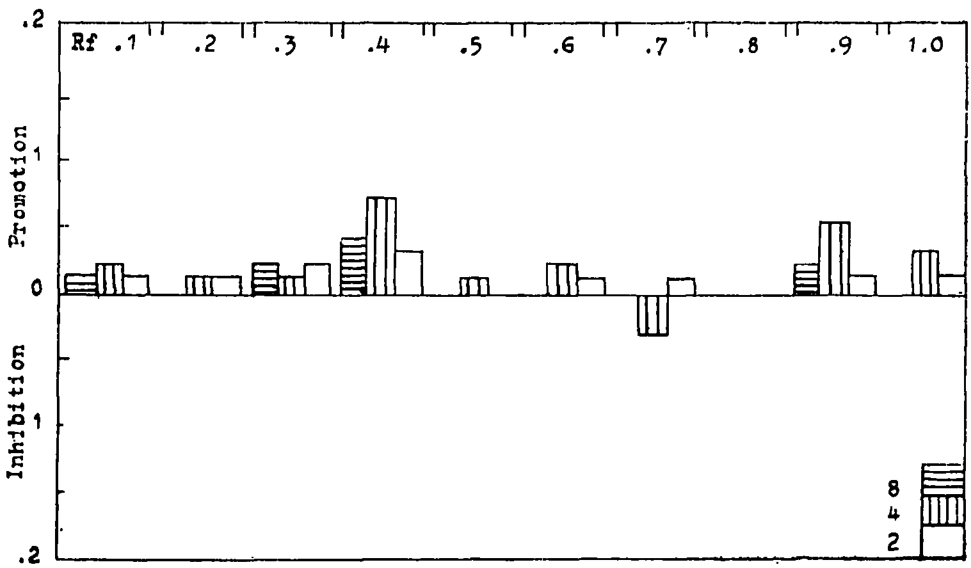

Figure 4. Growth substances present in the normal bark of *Hevea brasiliensis*. Extracted with ether and tested with *Mikania* at three concentrations (8,4,2). (Only the significant values are included. L.S.D. - 0.06 for 8 strips)

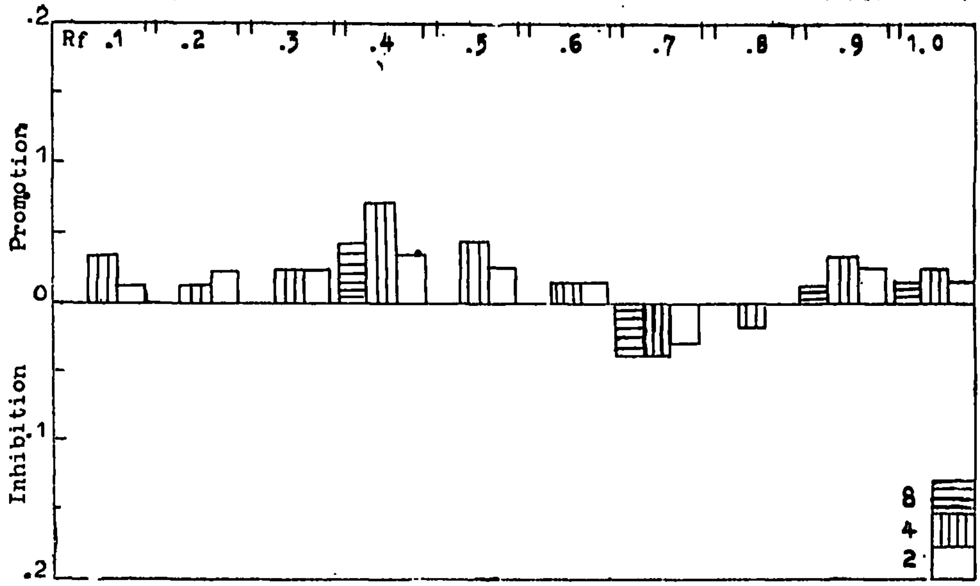

Figure 5. Growth substances present in the diseased bark of *Hevea brasiliensis*. Extracted with ether and tested with *Mikania* at three concentrations (8,4,2). (Only the significant values are included. L.S.D.=0.05 for 8 strips).

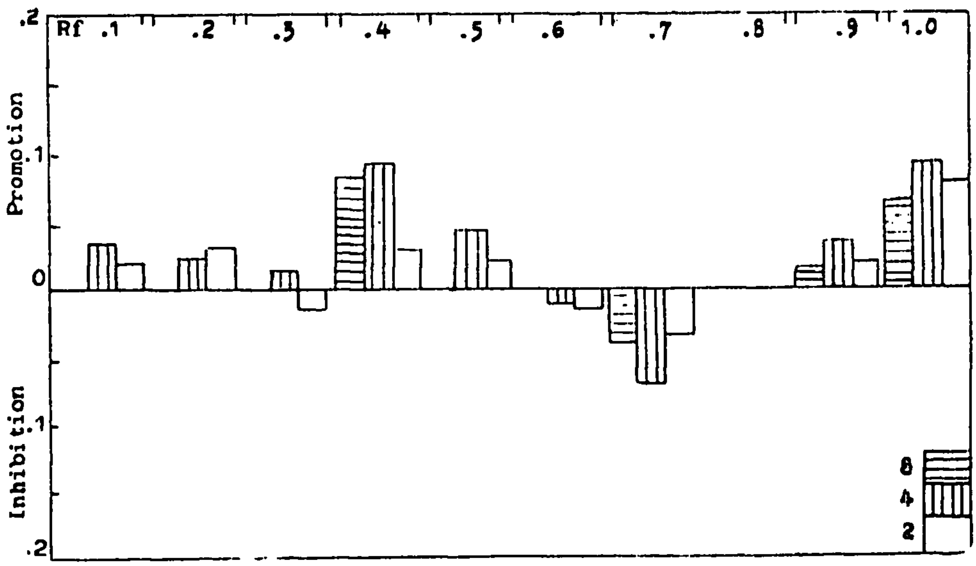

Figure 6. Growth substances present in the diseased bark of *Hevea brasiliensis*. Extract with ether and tested with *Coreopsis* at three concentrations (8,4,2). (Only the significant values are included. L.S.D.=0.06 for 8 strips).

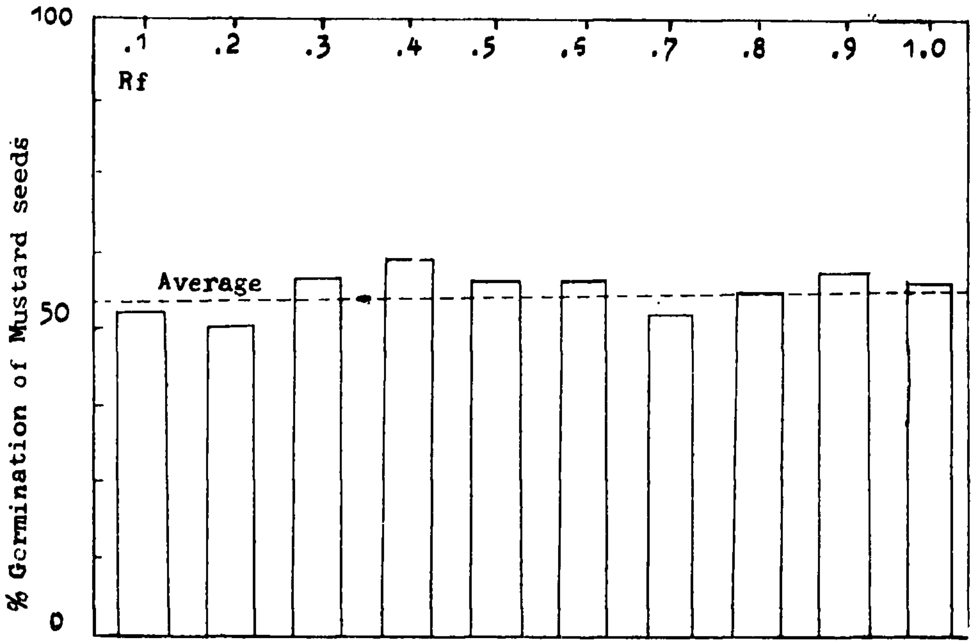

Figure 7. Percentage germination of ustard mseeds after 24 h. in diseased bark extract.

The effect on the rate of germination of mustard seeds was also tested. Two, four and eight samples of each Rf. were placed in the different petri dishes in order to assess the effect of possible concentration of the growth substances. As shown in Figs. 3 and 4 the best results are obtained with eight superimposed sections from equivalent portions of the strip. The histrograms at Rf. 4 and 1.0 indicated the presence of growth promoting substances. At Rf. 7 an activity inhibitory substance was observed. When mustard seeds were used to evaluate the effect on germination an inhibitory effect significant at 0.05% level was shown at the same position of Rf. 7.

Growth substances in seed oil, normal bark and diseased bark

Bark from a disease free tree of RRIC 36, and from a Brown Bast affected tree of the same clone was scraped off and dried in a vacuum oven at 35 °C for 48 h. The samples were then powdered using a Waring-Blender and equal quantities extracted with peroxide free ether at 45 °C for 30 h. in a Goldfish extractor. The solutions were concentrated by removal of most of ether, and spotted on Whatman No. 4 filter paper: the chromatograms were treated as for rubber seed oil and tested similarly.

RESULTS

The results obtained are shown in Figs. 4, 5, 6 and 7. As shown in Figs. 4 and 5 in both extracts there are growth promotors in similar positions in the paper. When tested with *Coreopsis* there appears to be no evidence of markedly growth inhibiting substances; however when the diseased bark (Fig. 6) was tested with *Coreopsis* there were growth inhibitors in positions 3, 6 and 7 and the highest concentration of inhibitory activity appears at Rf. 7 (position 7) as is the case for rubber seed oil.

When tested with mustard seeds for germination, as shown in Fig. 7, inhibitory substances are again demonstrated at Rf. 7 in the diseased bark samples.

Behrens test was applied to determine the significance of the inhibitory effect of the substances in the diseased bark. Analysis was however restricted to Rf. 0.7. The results indicated that when *Coreopsis* was used as the testing material the inhibitory effect was significant for 2, 4 and 8 superimposed paper strips: when *Mikania* was used the inhibitory effect was significant when 2 or 8 superimposed strips were used but was not significant when 4 strips were used.

DISCUSSION AND CONCLUSIONS

By comparing the growth substances in normal and diseased bark, it was observed, that the concentrations of growth promoting substances in both materials are similar. However, there is a slight variation in the growth inhibitory substances in the two. The results show that there is a higher concentration of growth inhibitory substances in the diseased bark. This was shown when tested with *Coreopsis* and *Mikania*, the difference being significant.

Comparing the growth substances (inhibitors and promotors) of rubber seed oil with bark extracts, growth substances appear to be clearly implicated in the phenomenon of Brown Bast. Concentrations of anti-auxins or inhibitors in rubber seed oil are higher than that of bark. Fernando & de Silva (1971) have shown an inverse correlation between oil content and growth of plants in *Hevea*, at least during the early period. Warring & Villers (1959) have observed this inhibitory substance in the region of 0.7 to 0.9 in extracts of *Fraxinus excelsier* seeds but the inhibitors develop only under moist conditions. Whether the same conversion is occurring in rubber seed oil is still not known. In *Fraxinus* this conversion of growth promotor to growth inhibitor takes place during the first 24—48 h. It has been shown that concentration of anti-auxins in the diseased bark is higher than in the normal bark. Whether this is the primary cause of the disease is not known. It may be that, this is one of the factors that lead to the diseased condition.

What makes this conversion of growth promotors to growth inhibitors is to be determined in connection with the high yielding property of *Hevea* trees. On the other hand it could be argued that the higher concentration of anti-auxins or the inhibitors observed in the diseased conditions is the result of the disease rather than the cause for it. Chua (1965) suggests that too frequent tapping leads to loss of enzymes, affecting the metabolic efficiency of the tissues. If this is operating through hormones, it may be that growth substances do take part in some role leading to the diseased condition.

Several studies have been made over the years to identify the cause of Brown Bast disease of the rubber tree. In our studies we have found that there is a significantly higher content of growth inhibitors in diseased than in healthy bark. However, it is not certain that this is the cause of the disease, and the studies are being continued. It is important to identify the cause, because high yielding clones are generally susceptible to Brown Bast, and it is quite possible that we are unwittingly breeding this character into high yielding clones. It would be possible to breed high yield Brown Bast resistant clones when the factor for susceptibility is indentified.

REFERENCES

ANON (1965). *Plr's Bull. Rubb. Res. Inst. Malaya* No. 80, p. 135.

BEAUFILS, E. R. (1954). Contribution to the study of mineral elements in field latex. *Proc. 3rd Rubb. Technol. Conf. London*, 87.

BOBILIOFF, W. (1925). Waarrimingen aan melksapraken in levenden toestant (Translation-Observations on living latex vessels). *Archs. Rubb. Cult. 1925.* **9**, 313 & 343.

CHAPMAN, G. W. (1951). *J. Rubb. Res. Inst. Malaya* **13**, 67.

CHUA, S. E. (1965). Physiological aspects of exploitation—tugor pressure. *Plrs' Bull. Rubb. Res. Inst. Malaya*. No. 80, p. 139.

COMPAGNON, P., TIXIER, P. & ROUJANSKY, G. (1953). Contribution a l'etude du accidents physiologiques de saignee. *Archs. Rubb. Cult.* Extra number. 1-54.

FERNANDO, D. M. AND DE SILVA, M.S.C. (1971). A new basis for the selection of *Hevea* seedlings. *Q. Jl. Rubb. Res. Inst. Ceylon.* **48**, 19-30.

FREY-WYSSLING, A. (1929) Microscopic investigations on the occurance of resins in *Hevea* latex. *Archs. Rubb. Cult.* **13**, 392.

RANDS, R.D. (1921a). Brown Bast disease of plantation rubber, its causes and prevention. *Med. Van. Let voor Plantenziekten* No. 47.

RANDS, R. D. (1921b). Histological studies on the Brown Bast disease of plantation rubber. *Med. Van. Let voor Plantenziekten.* No. 49.

RHODES, E. (1930). Brown Bast. Some considerations as to its nature. *J. Rubb. Res. Inst. Malaya.* **2**, 1.

RIDLEY, H. N. (1897). *Argic. Bull. Malaya Penins.* **7**, 136.

SCHWEIZER, J. (1949). *Archs. Rubb. Cult.* **26**, 345.

SCHWEIZER, J. COMPAGNON, P., & ROUJANSKY G. (1953). The physiology of latex as basis for tapping systems. *Archs. Rubb. Cult.* Extra number 1-54.

VOLLEMA, J. S. (1935, 1936 and 1937). *Voorlooplgs resullaten van de rubher toestuinen De Bercult.* **17**, 86-95.

WARRING, P. F. AND VILLERS, T. A. (1959). Growth substances and inhibitor changes in buds and seeds in response to chilling. Fourth International Conf. on Plant Growth Regulation held at Boyee Thompson Institute for plant Research in Yonkees., August 10-14.