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Ethylene Formation in Excised Hevea *Bark Discs*

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Data obtained in this investigation suggest that there is no clear correlation between wound ethylene formation in excised bark discs and plugging index both within a clone and between three clones studied. Nab 17, a clone very susceptible to wound damage, was found to evolve high levels of ethylene in excised bark discs.

There appeared to be no basipetal movement of ethephon from treated areas of the panel. Most movement appeared to be radial towards the cambium. This is in line with observations elsewhere that stimulants are most effective when applied close to the tapping cut.

Ethephon breakdown within the tissues seemed to be enhanced when bark discs excised from treated bark were sliced into smaller portions. Increased ethylene evolution was also observed when bark discs from auxin-treated strips were sliced.

The auxins 2,4-D, 2,4,5-T and NAA induced formation of significant amounts of ethylene in excised bark discs when applied at low concentrations, higher concentrations being ineffective. Copper sulphate was found to be ineffective at low concentrations, significant ethylene formation being observed only at very high concentrations. Acetylene did not induce any ethylene formation, suggesting that it acts independently of ethylene in delaying plug formation.

Formation of ethylene in plant tissues following injury or stress such as drought is a common and well-known phenomenon[1]. The practical significance of wound ethylene formation in *Hevea*, where exploitation procedures inevitably involve some form of injury is unknown. It is now apparent that ethylene is the common factor that is most likely involved in delayed plug formation following application of stimulants[2].

This study compares release of ethylene from bark discs taken from stimulated and unstimulated trees, in relation to some parameters. The effects of some chemicals, including some known yield stimulants, on ethylene evolution from bark discs are also described.

MATERIALS AND METHODS

The trees used in this investigation were those at the RRIM Experiment Station in Sungei Buloh. In each experiment, trees of the same girth, tapping history, clone and site were used.

A lanoline formulation of 1% α-napthaleneacetic acid (NAA) and commercial formulations of 10% ethephon, 1% 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) were used. These were applied by two methods: to the groove after removal of tree lace[3]; and, to scraped bark just below the tapping cut[4]. In all cases, unless otherwise stated, the stimulant was applied to the tree, 24 h before sample collection.

A weighed amount (5 g) of calcium carbide was applied to the tree by two methods: in a polystyrene applicator fixed to the tree 8 cm below the tapping cut[5]; and, to a bored hole at the stock-scion union[6]. Acetylene was generated by adding

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water to the calcium carbide at the two sites of application, 24 h prior to bark sampling.

Test chemicals were made up to the required concentration in 0.02 M Tris-HCl buffer at pH 6.0. For studies on the effects of the chemicals on ethylene evolution, 10 ml of the solution was added to each 130 ml flask containing the bark discs. For observations on browning which occurred at the cut edges and cambial surface, five bark discs were placed in 10 cm petri dishes containing 10 ml of the required test-solution. The discs were incubated in the dark at 25°C for 24 h and then observed and scored in arbitrary units in order of increasing discolouration as follows: 1, 1+, 2+ and 3+.

Bark discs were excised by driving a 1.2 cm cork-borer upto the cambium with a hammer. The discs were transferred immediately to 130 ml Quickfit conical flasks equipped with rubber septa in the glass stoppers to enable sampling of the air in the flasks. Generally, three to five discs were placed in each flask. In some experiments, bark discs were split into outer and inner halves and incubated separately. Sampling of the air in the flasks was carried out with plastic syringes equipped with hypodermic needles. Samples of 1 ml were taken at 24 h intervals, generally for upto five days.

Ethylene analyses were carried out by gas-chromatography using a Hewlett Packard Model 7620 instrument fitted with a 183 × 0.317 cm column packed with Duropak OPN (mesh size 80–100) and operated at 60°C. The carrier gas was N_2 used at a flow rate of 28 ml per minute. A H_2-flame ionisation detector was used.

The peak heights on the recorder traces were measured together with the peak height of a standard made up of 100 p.p.m. of ethylene in nitrogen. The concentration of ethylene in the flask was worked out using the following formula:

$$\text{p.p.m. } C_2H_4/\text{g tissue} = \frac{\text{sample peak height}}{\text{standard peak height}} \times \frac{100 \times \text{volume of flask}}{\text{weight of sample}} \times \frac{\text{attenuation of sample}}{\text{attenuation of standard}}$$

Plugging index was determined according to Milford *et al.*[7], during the month of September. The mean of three determinations was used as representative of an individual tree.

Results given for each experiment are representative of at least two determinations unless otherwise indicated.

RESULTS

Preliminary Observations

Bark discs were sampled 2.5 cm below the tapping cut from six-year-old buddings of GT 1 and incubated in sealed conical flasks as described earlier. Ethylene release was monitored for upto seven days *(Figure 1)*. After an apparent lag phase

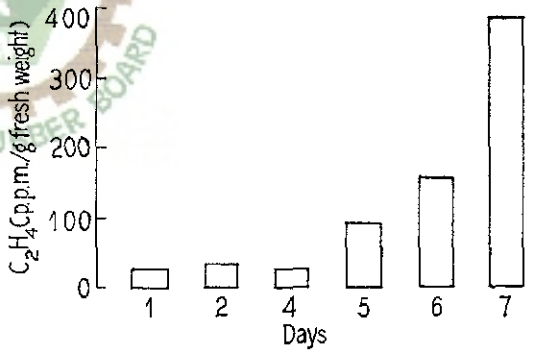

Figure 1. Evolution of ethylene from bark discs excised from mature GT 1 unstimulated trees.

of four days, there was progressive increase in ethylene content of the flasks in which the discs were incubated, suggesting continuous formation during the period of observation.

Comparison of ethylene evolution from bark discs taken from various clones is shown in *Figures 2* and *3*. The relatively high evolution of ethylene from bark discs of Nab 17 *(Figure 2)* which is generally regarded as a wound susceptible clone, is

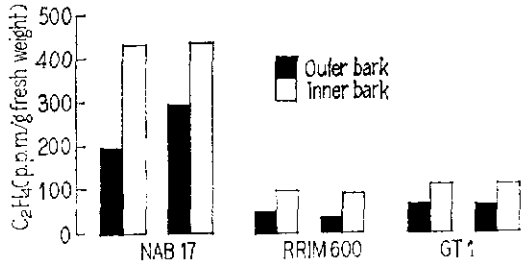

Figure 2. Ethylene evolution from bark discs sampled 2.5 cm beneath the tapping cut on virgin bark of two mature trees each of NAB 17, RRIM 600 and GT 1. Ethylene evolution was determined five days after excision. Bark discs were split into outer and inner equal halves and incubated separately.

to be noted. Where three clones representative of high, medium and low plugging index values are compared *(Figure 3)*, it is evident that higher ethylene evolution was from PR 107 which is known to be a high plugger[7]. Bark discs from the medium (RRIM 612) and low plugging (RRIM 513) clones showed approximately equal evolution of ethylene. The difference in ethylene evolution *(Figures 2* and *3)* between inner and outer halves of bark discs could probably be due to variation in panels sampled, one being virgin and the other being renewed bark. These preliminary observations suggested clonal differences, which however, could not be related to plugging index. The same was evident when ethylene

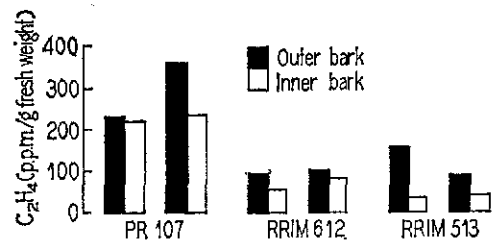

Figure 3. Ethylene evolution from bark discs sampled 2.5 cm beneath the tapping cut on renewed bark (first renewal) from two mature trees each of PR 107, RRIM 612 and RRIM 513. Ethylene evolution was determined five days after excision. The bark discs were split into outer and inner halves and incubated separately.

evolution from bark discs from trees with varying plugging index values within a clone was examined *(Figure 4)*.

Ethylene evolution from discs varied with the position of the bark on the tapping panel from which they were excised. In an experiment in which bark discs were excised at various points on a vertical line along the

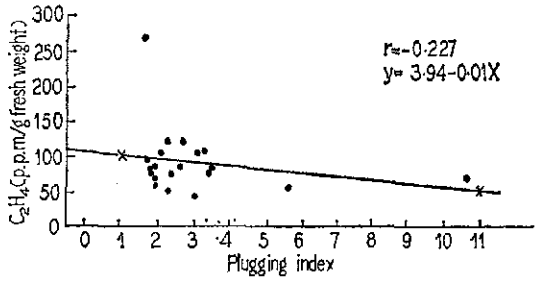

Figure 4. Variation in ethylene release in relation to plugging index in clone RRIM 628. Bark shavings were used for this study. The mean of three determinations of plugging index made at weekly intervals during the month of September, is plotted against the mean of three determinations of ethylene release from bark shavings, also collected at weekly intervals. Ethylene evolution was determined five days after incubation.

hole, it appeared that evolution of ethylene was greater from bark discs excised closer to the union between trunk and rootstock *(Figure 5)*. Evolution of ethylene was also greater in bark discs from regenerating bark immediately above the tapping cut than in bark discs from a position immediately below the tapping cut. There was also evidence that evolution of ethylene from bark discs in virgin bark above the regenerating panel of bark was higher than in bark discs from a position 5 cm below the tapping cut.

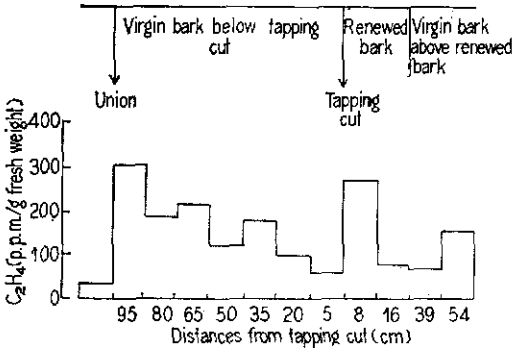

Figure 5. Ethylene evolution after five days of incubation from GT 1 bark discs excised at various vertical distances from the tapping cut of mature trees.

Ethylene Release in Ethephon Stimulated Trees

Bark discs were sampled 120 h after stimulation at three positions: 1 cm below the tapping cut at a height of 152 cm from the union; mid-point of the panel; and, immediately above the union. Whole bark discs, as well as bark discs sliced into three approximately equal outer, middle and inner portions, were incubated separately.

As expected, ethylene evolution from bark discs taken from the scraped site of application of ethephon was exceptionally high *(Figure 6)*. Evolution of large quantities of ethylene from inner and middle portions of bark discs suggested lateral movement of ethephon into the bark tissues. In bark discs taken from trees stimulated by the groove method, ethylene evolution was not different from bark discs taken from unstimulated trees, suggesting insignificant basipetal movement of ethephon.

Bark discs taken either at the midpoint level between the tapping cut and union or near the union showed significantly less ethylene evolution, suggesting relatively poor basipetal translocation of ethephon. The levels of ethylene evolved from the latter two types of bark discs were comparable with that evolved from bark discs from unstimulated trees.

It was also evident that total ethylene evolution from the three sliced portions was much greater than that of the unsliced whole bark discs. It seems probable that slicing enhanced breakdown of ethephon within the tissues *(Figure 7)*.

In trees stimulated with acetylene using calcium carbide, bark discs taken either at the tapping cut or above the stock-scion union did not show increased levels of ethylene evolution in relation to controls.

Effects of Other Stimulants

Ethylene evolution in excess of control discs was evident in bark discs excised from the treated area of trees stimulated with 1% 2,4,5-T, 2,4-D or NAA. The increase in evolution of ethylene following slicing was also evident in discs treated with auxins. With 2,4,5-T and NAA treatment, ethylene evolution was greatest from the outer portions, whereas with 2,4-D treatment, the inner half appeared to be as active as the outer half in evolution of ethylene *(Figure 8)*.

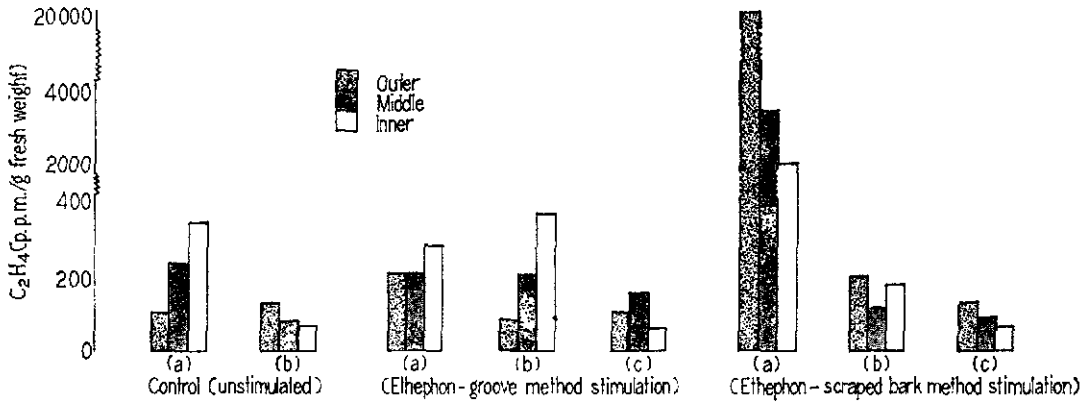

Figure 6. Ethylene evolution after five days' incubation from bark discs from mature trees of LCB 1320. Bark discs were from (a) 1 cm beneath the tapping cut; (b) mid-point of the panel between the tapping cut and union; and, (c) 2.5 cm above the union.

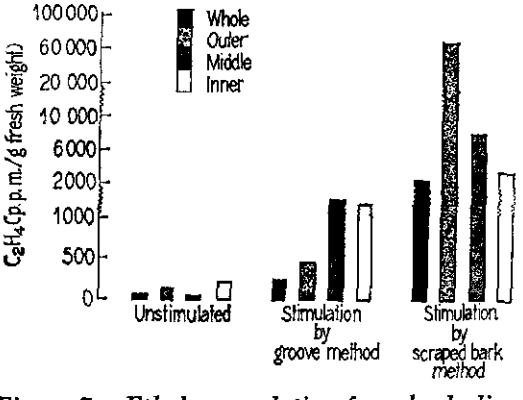

Figure 7. Ethylene evolution from bark discs excised from stimulant-treated bark of LCB 1320 trees. Ethylene evolution from whole bark discs is compared with that from sliced portions. Ethylene levels were determined after five days of incubation.

Effects of Various Chemicals on Ethylene Evolution from Excised Bark Discs

Selected chemicals were tested for their effect on ethylene release over a range of concentrations *(Figure 9)*. Napthaleneacetic acid was effective in significantly promoting ethylene evolution at 10 p.p.m. and slightly at 100 parts per million. Higher concentrations of NAA were ineffective in promoting ethylene evolution. Trichlorophenoxyacetic acid promoted ethylene evolution at 10 p.p.m. while higher concentrations appeared ineffective. Copper sulphate was ineffective at concentrations upto 1000 p.p.m.; while at 1000 p.p.m., the effect on ethylene evolution was clearly significantly large. Kinetin, gibberellic acid, chloramphenicol, cycloheximide and calcium sulphate were all ineffective at the concentrations used as indicated in *Figure 9.*

Untreated bark discs developed a brownish discolouration on the cut edges as well as the cambial surface during incubation. This was evident within a few hours of excision. Ethephon-treated discs did not develop the brownish discolouration. High concentrations of 2,4,5-T or NAA (1000 p.p.m. or 2000 p.p.m.) and chloramphenicol or cycloheximide (1000 p.p.m. or 2000 p.p.m.) also appeared to prevent the development of the brownish discolouration. It appeared from these observations that ethylene release was not the causative factor involved in prevention of browning by ethephon as discolouration was also prevented by chemicals at concentrations which were ineffective in promoting ethylene evolution.

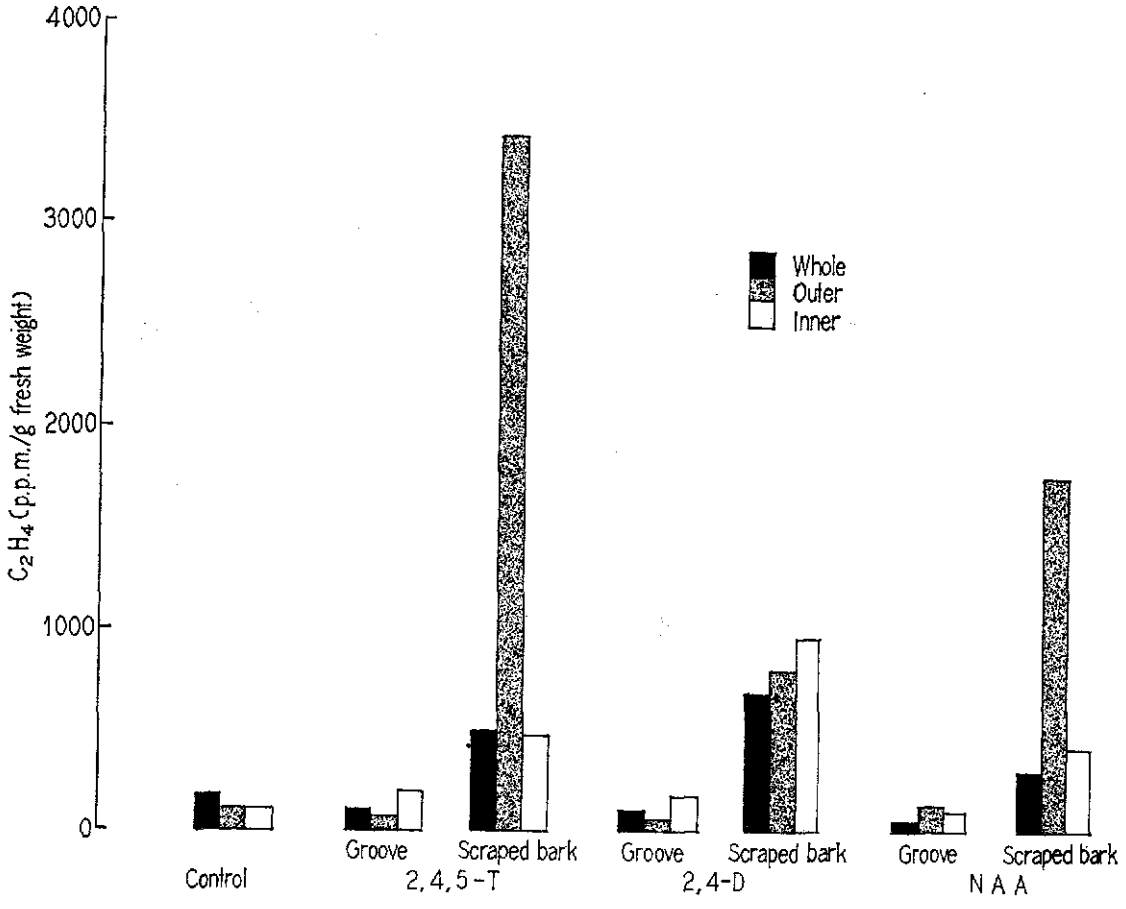

Figure 8. Ethylene evolution from bark discs excised from stimulant-treated bark of mature trees of RRIM 628. Ethylene evolution from whole bark discs is compared with sliced portions. Ethylene levels were determined after five days of incubation.

DISCUSSION

All known exploitation procedures in *Hevea* entail some form of injury to the bark. The excised, and therefore injured bark discs, were found to form ethylene for prolonged periods and it is likely that wound ethylene is also formed as a result of injury caused by tapping. While it appears generally accepted that ethylene is involved in some way in delay of plug formation[8], the role of wound ethylene in this process is unknown. The present study indicates that wound ethylene is not likely to be of significance in prolonging latex flow as there appeared to be no correlation between plugging index and ethylene formation in excised bark discs. Furthermore, ethylene formation appeared to be lowest in bark discs collected close to the tapping cut.

The evolution of relatively large levels of ethylene from excised NAB 17 discs suggests that severity of wound reactions may be associated with significant ethylene formation. The clone NAB 17 is known to frequently express wound reactions following deep tapping in the form of bark fissures

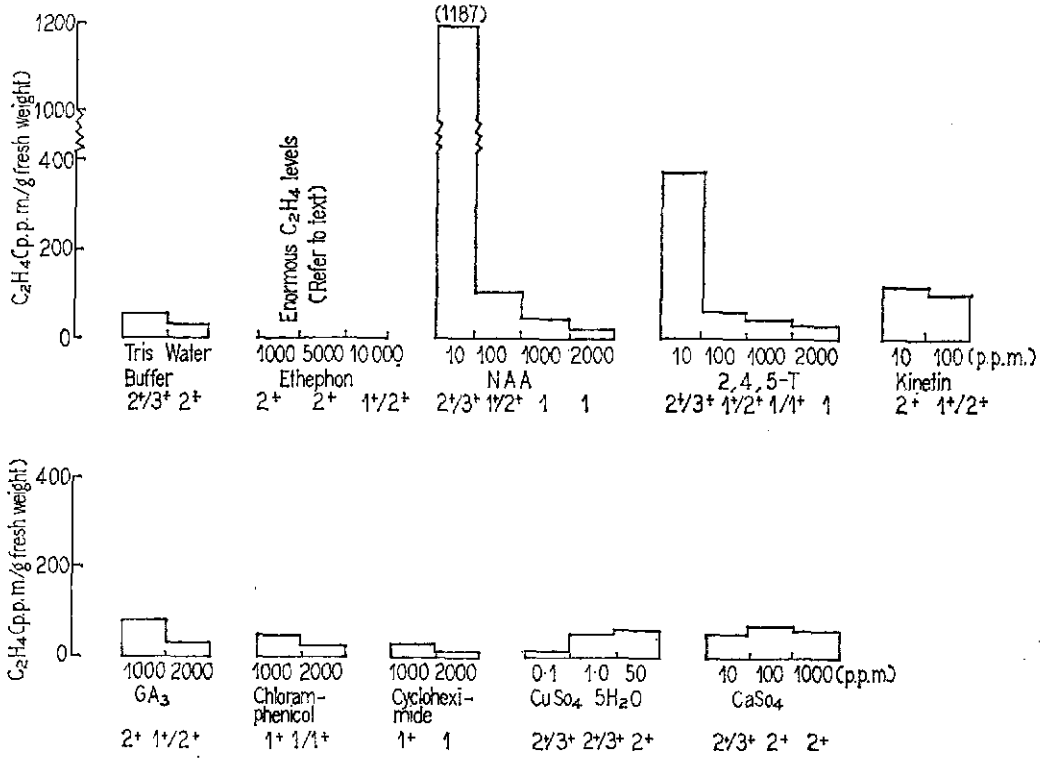

Figure 9. Effect of various chemicals on ethylene evolution and visual scores on browning of bark discs excised from mature RRIM 705 trees in tapping. Ethylene was determined after five days' incubation.

and bark protuberances[9]. Puncture tapping involves injury of the cambium and in some clones, depending on the age of the trees, the wound reaction may be severe. It would be of interest therefore, to determine if differences in wound ethylene formation could be related to differences in wound reactions between clones in puncture tapping or deep tapping.

The increasing evolution of ethylene from samples taken closer to the union is worthy of note. A plausible explanation might lie in an increasing juvenility down the bole. Hormonal changes with respect to juvenility have been demonstrated[10] and it would be of interest to ascertain a similar relationship with respect to wound ethylene formation in *Hevea* where a gradient of juvenility along the bole is a well-known feature. Nevertheless, other factors such as active bark regeneration or intensity of latex drainage may be involved as well. Thus, bark discs immediately above the tapping cut from regenerating bark showed high levels of ethylene evolution. Bark discs from virgin bark above the regenerating panel also showed high levels of ethylene evolution.

Samples collected from trees treated by the scraped bark method with ethephon, 2,4,5-T, 2,4-D or NAA showed ethylene evolution only when the samples were from the site of application. Samples from trees stimulated by the groove method showed low levels of ethylene evolution even when these were about 5 cm from the tapping cut.

Basipetal movement of the stimulants, as revealed by ethylene evolution from bark discs, is evidently poor. Lateral movement of stimulants into the bark at the site of application is very significant as indicated by the levels of ethylene evolution from the inner portions of bark discs. It is likely, therefore, that stimulant action is localised to the site of application. Stimulants are most effective when applied close to the tapping cut[11] and it is plausible that their effect in delaying plug formation arises from some change induced at the tapping cut itself. Latex coagulants have been demonstrated in bark extracts of *Hevea*[12] and it was also shown in the same study that extracts from stimulant treated bark had less flocculating potency on latex when compared with control trees. Another factor that probably is involved in aiding the optimal effect of stimulants at sites close to the tapping cut is the tapping injury induced release of ethylene in stimulant-treated bark. The increased release of ethylene following slicing of bark discs was evident not only in samples from ethephon-treated bark but also in samples from auxin-treated bark. It would be of interest as a further extension of this study, to investigate the extent of ethylene release in untapped auxin-treated bark and in tapped auxin-treated bark *in situ*. In some exploitation experiments, it has been observed that ethephon effects on yield stimulation were evident a year or even later when the treated strip was finally tapped. It is thus likely that ethephon breakdown in uninjured bark is slow but is enhanced by tapping.

Audley *et al.*[13] in their investigations on translocation of ^{14}C-ethephon in two-year-old seedlings indicated that their observations were consistent with an initial upward movement of ethephon in the transpiration stream. In the present study, ethephon movement above the tapping cut was not considered. Any movement of ethephon below the tapping cut appeared insignificant. The use of ^{14}C-ethephon may lead to a firmer confirmation of the present observations in mature tapped trees.

Audley *et al.*[14] have made a comprehensive study of the effects of a variety of chemicals on ethylene formation in leaf discs and stem segments of *Hevea*. The authors have attempted to extend their findings by application of selected substances to bark discs excised from mature trees. Auxins like 2,4,5-T, NAA and 2,4-D were all effective in inducing ethylene evolution at a low concentration of 10 p.p.m. each. Higher concentrations of 100 p.p.m. and 1000 p.p.m. were clearly ineffective. Thus, in commercial field applications which involve 1% concentrations, it is likely that only low levels of the auxins diffuse into the bark tissues. In contrast to the auxins, copper sulphate was ineffective at low concentrations upto 100 p.p.m. but effective at 1000 p.p.m. in inducing ethylene evolution. Its effectiveness at high concentrations only may explain the requirement for bore hole applications as it is generally ineffective when applied onto scraped bark.

Acetylene released in the tree using calcium carbide did not appear to induce any ethylene formation in bark tissues. This indicated that acetylene was acting directly in delaying plug formation unlike other stimulants which either released ethylene or induced formation of ethylene in the tissues.

In studies where excised bark discs were incubated with ethephon, browning appeared to be inhibited. It was of interest, therefore, to investigate if this property of inhibition of browning was a feature of yield stimulants generally. This however, appeared unlikely as the auxins like 2,4,5-T and NAA were found to inhibit browning only at the high concentrations at which

ethylene evolution was not enhanced. At low auxin concentrations at which ethylene evolution was significant, browning was not prevented. Inhibitors of protein synthesis like chloramphenicol and cycloheximide seemed to inhibit browning without any enhancement of ethylene evolution. Both these compounds do not act as yield stimulants[15]. Thus, the effect of most yield stimulants on browning may be an effect unrelated to their effects on plugging, which most likely is delayed by ethylene arising after stimulant application.

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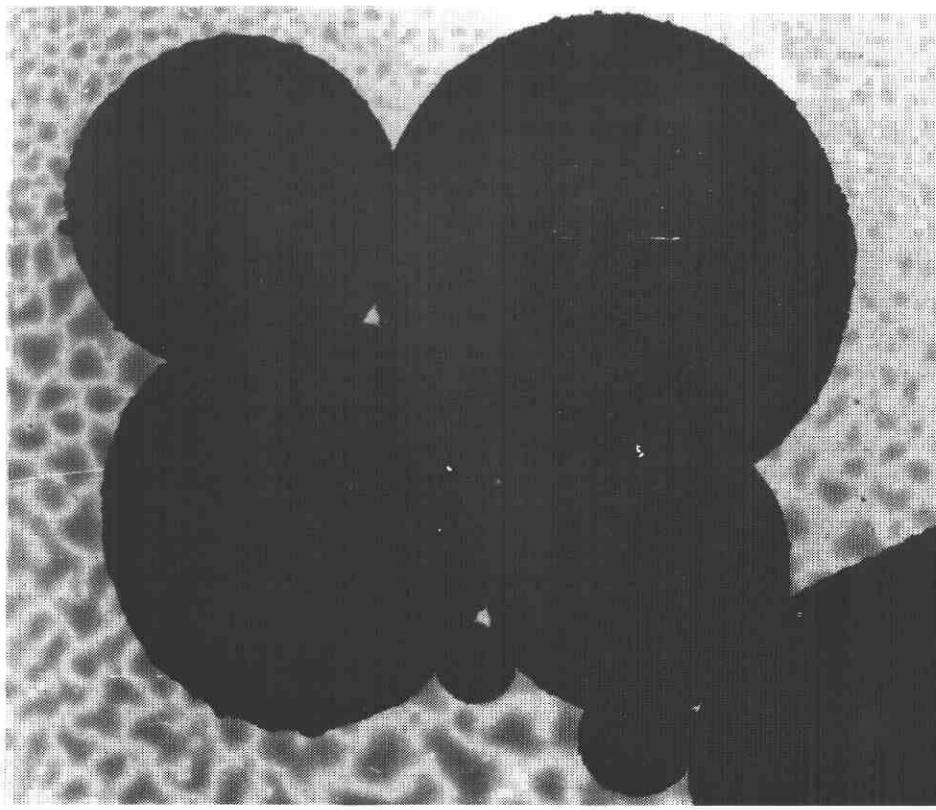

Figure 5. Whole-mount electron micrograph of MG 49 powder (4158 X).

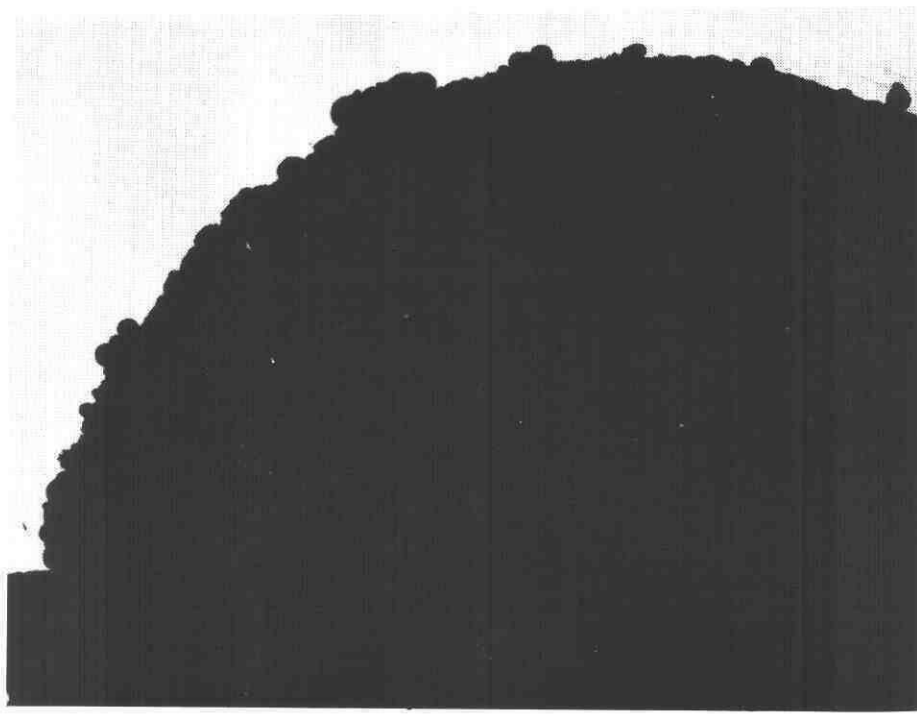

Figure 6. Whole-mount electron micrograph of MG 49 powder (26 730X)

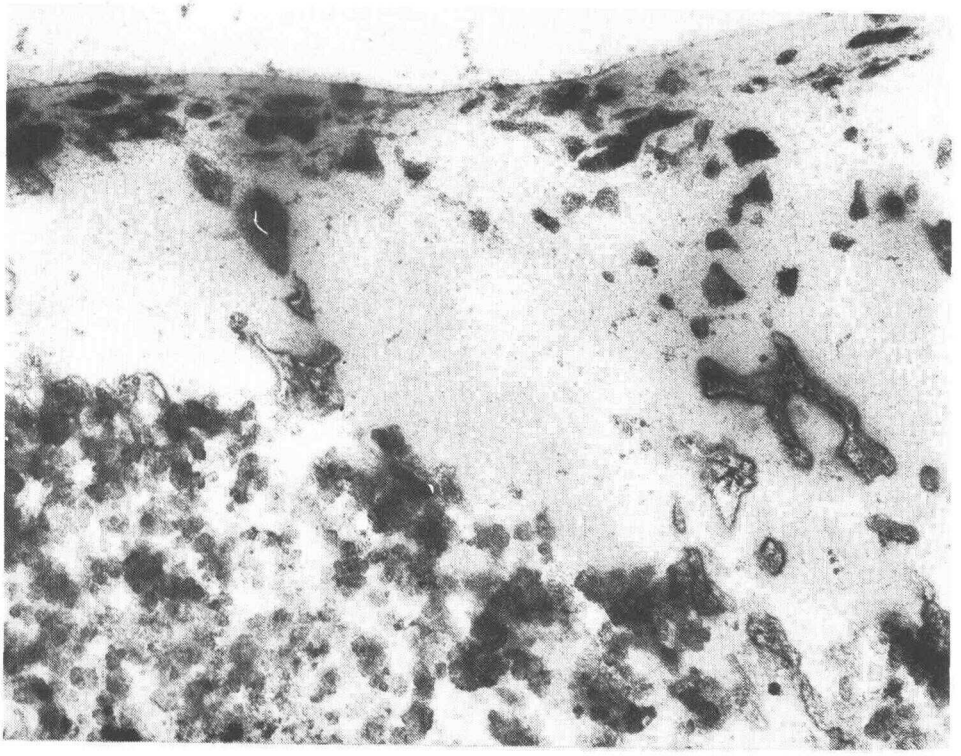

Figure 3. Sectional electron micrograph of agglomerated natural rubber powder (52 000X).

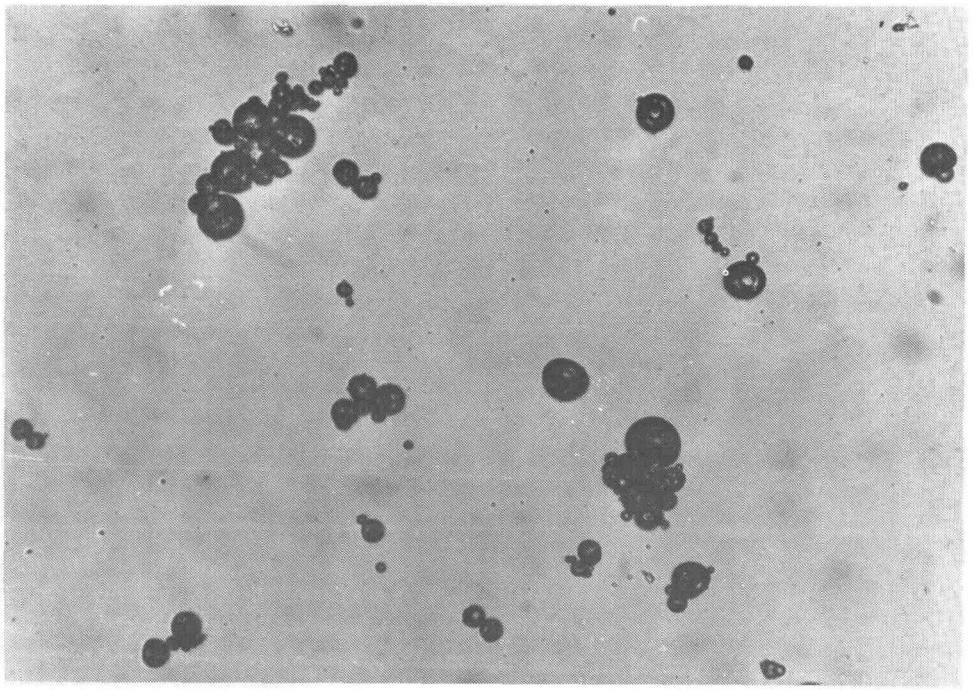

Figure 4. Optical micrograph of MG 49 powder (100X)