J. Rubb. Res. Inst. Malaysia, **29(2)**, 86 – 95

Anatomy of Bark Renewal in Normal Puncture Tapped Trees

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The process of bark renewal in normal puncture tapped trees was examined by light microscopy. The anatomy at the punctured point revealed a temporarily damaged vascular cambium where regeneration of the bark soon proceeded smoothly as in the normal process of wound repair. Latex vessel initiation however, was at first delayed as shown by the gap in the first two latex vessels in the newly formed phloem. The darkened scab tissue or 'wounded zone' seen on removal of the flaky external bark was actually part of the bark which reacted to the puncturing, producing some chemical substances. Renewal in mini-drilled bark also took a similar course except that the damaged zone in the inner bark was closely confined to the puncture.

Puncture tapping in *Hevea*, known as pricking was first reported by Wright[1] in 1906. It was not until 1965 that further description of the method was made when Lustinec and Resing[2] studied the drainage area. Unlike the conventional tapping system where the principle of excision is used, puncture tapping or micro-tapping involves simple needle incisions made on a strip or on a shallow groove of bark, usually previously stimulated.

The French[3,4,5] revived the work on microtapping recently, mainly because of their interests in obtaining latex with as little disturbance as possible to the bast tissue, as they have shown previously that tapping by excision and stimulation reduced the yield and the sugar content in latex. Tupy and co-workers[3,4,5], in contrast to workers in RRIM[6] and Thailand[7], found a significant increase in the sucrose content in latex from micro-tapped trees. The discrepancy of results obtained from studies of sucrose content in latex of micro-tapped trees has been given by Low[8].

However, yield responses and bark reactions to microtapping systems are of more concern particularly in view of the growing shortage of skilled labour in rubber plantations. Many studies pertaining to microtapping systems e.g. number of punctures, length or strips and types and ages of materials used have been carried out widely[9,10,11]. These initial studies reported encouraging yield performances. Different types of semi- or fully automated puncture-tapping tools have been designed and evaluated in field trials[12]. An alternative to puncture tapping, the micro-X method was also introduced[13]. This method incorporates a higher intensity of microtapping followed by a reduced number of conventional tappings prior to re-stimulation. Ismail *et al.*[13] in their initial study indicated better yield performance with the micro-X method than the microtapping system.

Despite its desirable features, punture tapping has its shortcomings. Three forms of bark reaction to puncture tapping both on renewed and virgin bark have been identified[13,14]. Widely observed in all puncture-tapped trees is the external flaking of the unproductive tissue consisting of cork cells and peridermal tissues, the removal of which exposes the pitted

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surface. Development of the flakes may have been due to the reaction of the bark to yield stimulants. Although pitted bark appears unattractive, it yields when re-pricked as the cambium is not permanently damaged.

Bark bursts which look like 'patch cankers' are restricted only to certain clones identified as wound-susceptible and in particular on young mature and immature trees. No pathogens have however been identified to be involved in these bark bursts. The third form of bark reaction, the uneven bark swellings or bulges are only noticeable in certain clones like RRIM 600, RRIM 703 and PB 28/59. This problem is therefore not common and in the cases examined, it appears to be a reaction to premature and excessive multiple injury.

Some aspects of the regenerated bark after puncture tapping have been discussed by Tonnelier *et al.*[15] Only a photomicrograph of the callus formed around the damaged portion caused by microtapping has so far been given[16]. This paper examines the process of bark renewal in normal puncture-tapped trees and estimates the damage caused by puncture tapping. Renewal of bark tissue from wounds produced by a mini drill is also discussed.

MATERIALS AND METHODS

Bark samples were taken from trees which had been puncture-tapped for six months to three years. The trees chosen showed typical bark renewal of puncture-tapped trees.

A 2 cm circular plug was removed from each puncture point to which occasionally the wood was also attached. The samples were fixed in formalin acetic alcohol (FAA), hand-sectioned and stained with either oil red 'O' or oil blue NA. Slides prepared were examined under the light microscope and coloured micrographs were taken.

OBSERVATIONS

Structure of Renewing Bark of Tapped Surfaces

In conventional tapping by a skilled tapper, the bark is excised up to 1 mm from the cambium. The exposed cells of the phloem, which also consist of a few rows of latex vessels, are immediately sealed with fatty substances including suberin[17]. This blocking seems to create favourable internal conditions for meristematic activity of the cork cambium (phellogen). A corky layer consisting of suberised cells on the outside (phellem) and parenchyma cells on the inside (phelloderm) are formed through the activity of the cork cambium. Together, the new tissue formed is known as the periderm and is unproductive as far as latex production is concerned.

Meanwhile, the vascular cambium too divides, forming the phloem of the renewing inner bark and also the xylem tissues. Activity of the vascular cambium is normally associated with secondary thickening but in this instance, activity is accelerated by wound reaction after tapping. Two to three latex vessel rings per year is the average rate of initiation in a normal phloem tissue but regenerated bark, e.g. a two-and-a-half-year-old bark, completely resembles the original bark as claimed by Bobillioff[17]. The latex vessels that are retained in the untapped bark are gradually displaced outwards as new phloem tissue is produced.

Process of Bark Renewal in Puncture-Tapped Panel

As the bark is punctured, the wound is closed by a plug of latex which probably has a protective function. The cells adjacent to the puncture too seem to be affected as shown by the discolouration, which is also more intense along the periphery of the wound *(Figure 1)*. In sections which are previously bleached prior to staining, the discolouration is removed except in the more intensely discoloured zone exposing clearly the latex vessels *(Figure 2)*. This indicates that the 'wounded-zone' is still a part of the bark and the discolouration is due to the presence of substances perhaps protecting the protoplasts against decay or desiccation. The more intensely discoloured zone, on the other hand, is made up of lignified/suberised cells as shown from the thin sections prepared for electron microscopy but examined under the light microscope *(Figure 3)*. This was however identified as tannin cells by Tonnelier *et al.*[15] Some cells with lignified/suberised cell walls are also shown.

Cork cambium is presumably developed from the peripheral cells of the 'wounded-zone'. The peridermal tissue is formed and the phelloderm later develops into stone cells. *Figure 4* shows the formation of stone cells only below the 'wounded-zone' a year after puncture tapping. Three years from the time of wounding the stone cells completely surround the 'wounded-zone'

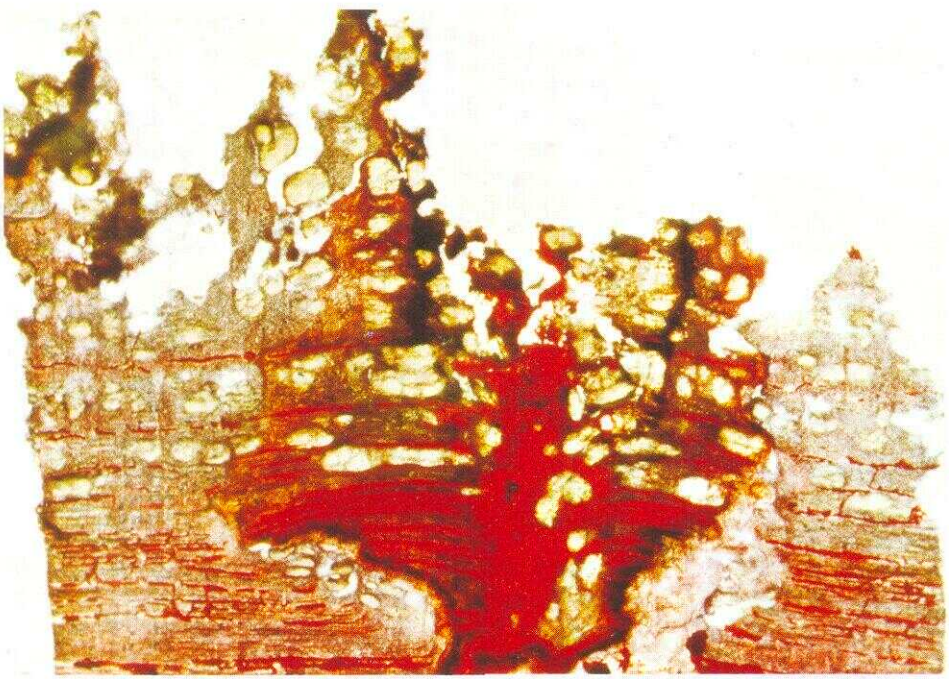
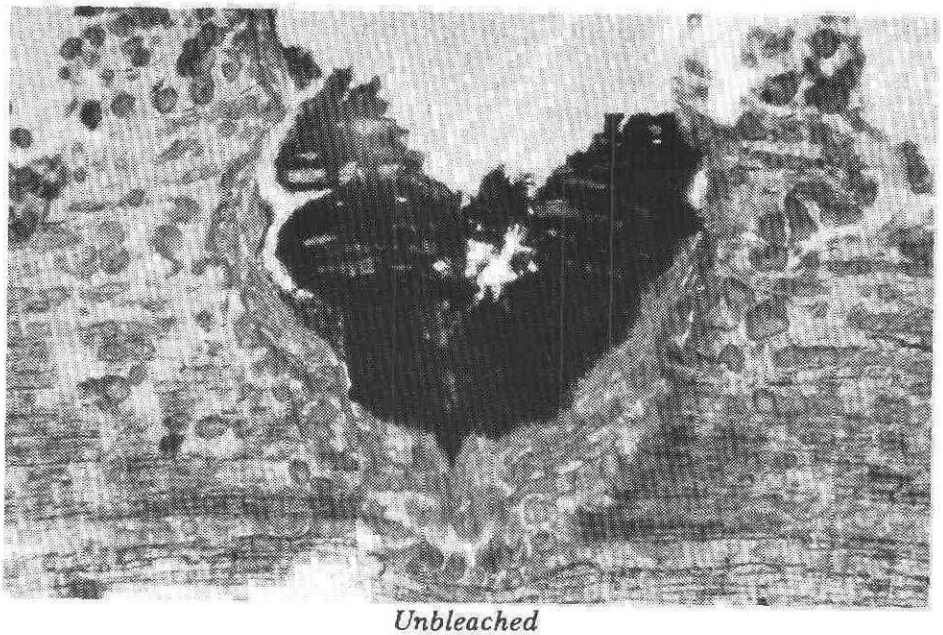
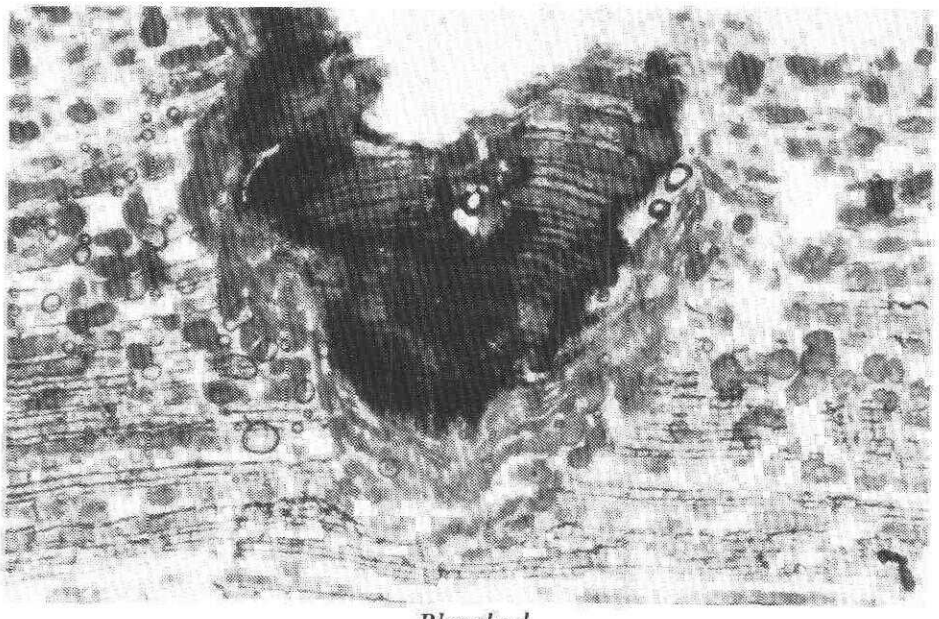


Figure 1. Longitudinal section of six-month-old puncture-tapped RRIM 703 bark. Note the discolouration of tissue surrounding the latex plug (p) . . Magnification 12 ×.



Unbleached



Bleached

Figure 2. Comparison of unbleached and bleached longitudinal sections of three-and-a-half-year-old puncture-tapped PR 107 bark. Bleaching removes the discolouration in the 'wounded zone' exposing clearly the latex vessels. Magnification 12×.

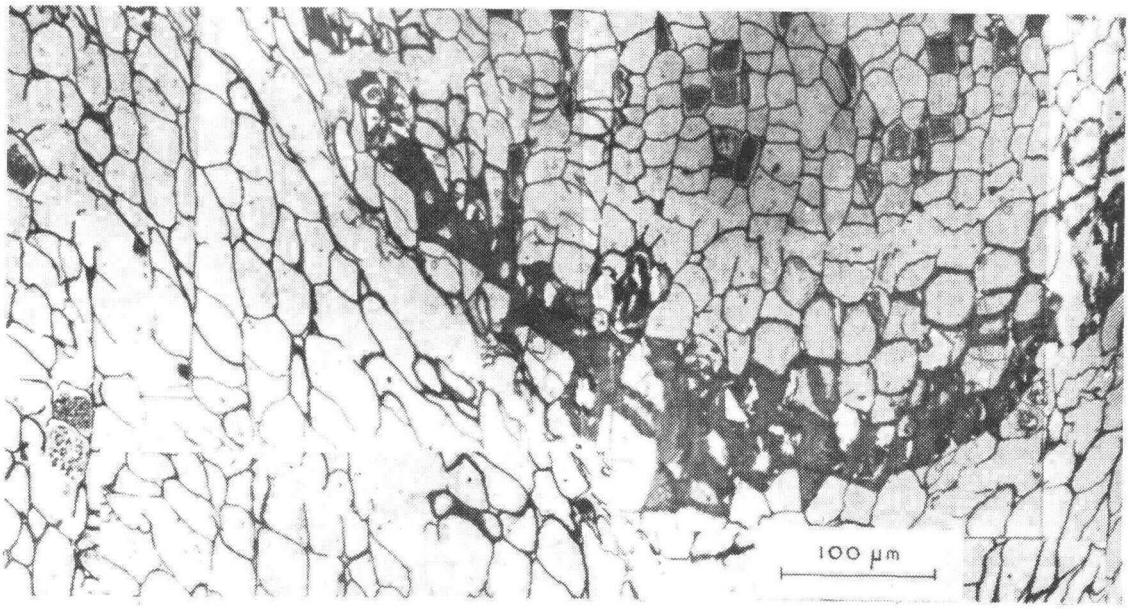


Figure 3. Longitudinal section of three-month-old puncture-tapped PR 255 bark showing lignification/suberisation of cells along the periphery of the 'wounded zone'. Note the thickening along the cell walls. Stained with toluidine blue. Magnification 220 ×.

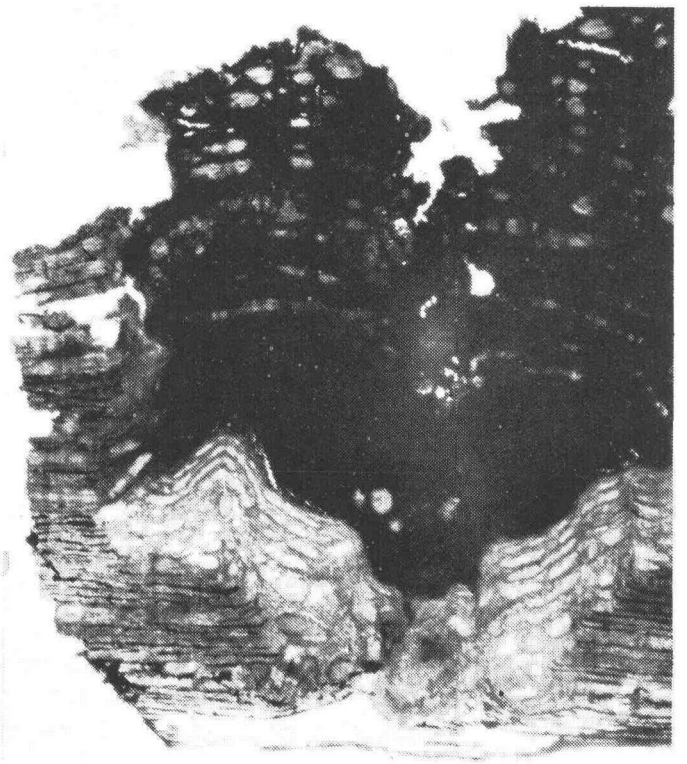


Figure 4. Longitudinal section of one-year-old puncture-tapped PR 107 bark. Note the formation of stone cells below the 'wounded zone'. Magnification 12 ×.

(Figure 5). The massive formation of stone cells which appear to be aligned in rows, have by now also displaced the zone outwards.

The scar tissue or scab seen on the surface of the bark when the flaky external bark is removed *(Figure 6)* is actually made up of the 'wounded zone' plus the peridermal tissue and some of the stone cells. The scar tissue or scab detaches easily especially during sectioning and this is probably due to the actively growing cork cambium present.

The continuity of the vascular cambium in the meantime is not affected permanently by the puncture; bark renewal from the vascular cambium proceeds in the normal fashion. Latex vessel initiation in the newly formed phloem seems to be delayed at first and is seen as a gap between the first two latex vessels near the cambium *(Figure 5)*; after which the formation of latex-vessels appears normal again. The extent of formation of phloem tissue depends on the age of renewal.

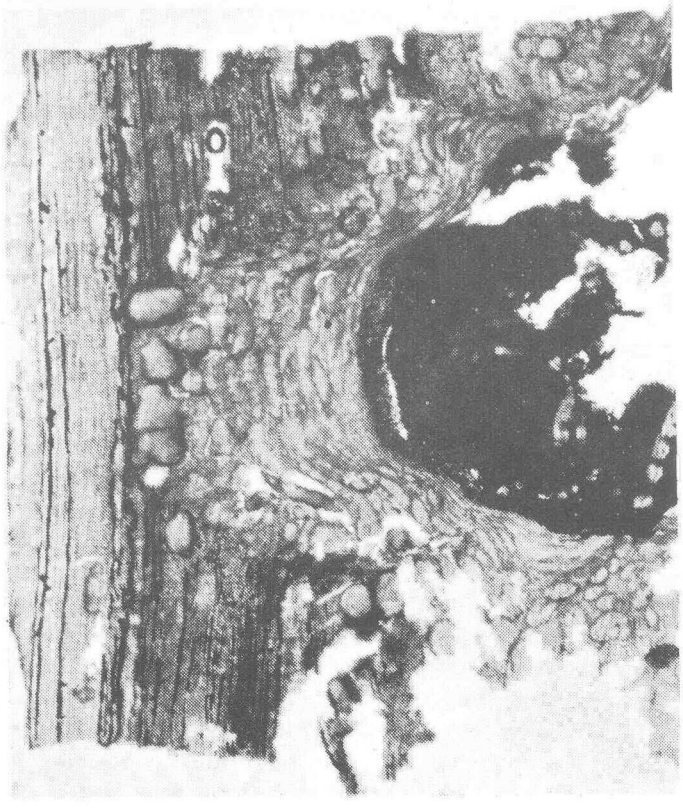


Figure 5. Longitudinal section of PR 107 bark showing the healed wound three-and-a-half years after puncture tapping. Stone cells completely surround the 'wounded zone'. Note the gap between the first two latex vessels in the newly formed phloem. Magnification 12 X.

Figure 6. Scar or scab tissue on the surface of RRIM 600 bark below the flaked periderm, two years after puncture tapping.

In a wound produced by a mini-drill, the 'wounded zone' as compared to that produced by ordinary puncture tapping by other forms of needles is closely confined to the puncture and appears rather regular (compare *Figures 7* and *1*). Similar cork cambial activity takes place here too. The stone cells that usually accompany the phelloderm are however less conspicuous especially in the inner bark even eighteen months after puncturing *(Figure 8)*.

The type of bark renewal as described above, exposes wood that is smooth surfaced when the bark is removed. In some clones however, punctured points stimulate meristematic activity at the cambium producing nodules which can only be seen when the bark is removed. The wood in this case has grown into the puncture-tapped wound (*Figure 9*). In extreme cases when these nodules project into the bark, obstructions are created in the bark.

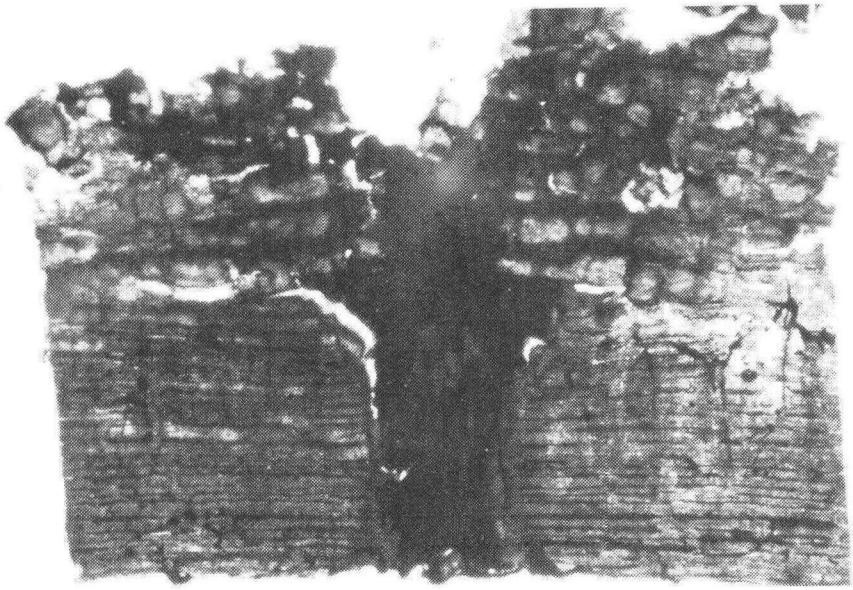


Figure 7. Longitudinal section of RRIM 701 bark showing the wound produced by a mini-drill six months after puncture tapping. The 'zone' is closely confined to the puncture. Magnification 12 X.

Figure 8. Longitudinal section of RRIM 701 bark showing an eighteen-month-old wound produced by a mini-drill. Note the paucity of stone cells surrounding the 'wounded zone'. Magnification 12X .

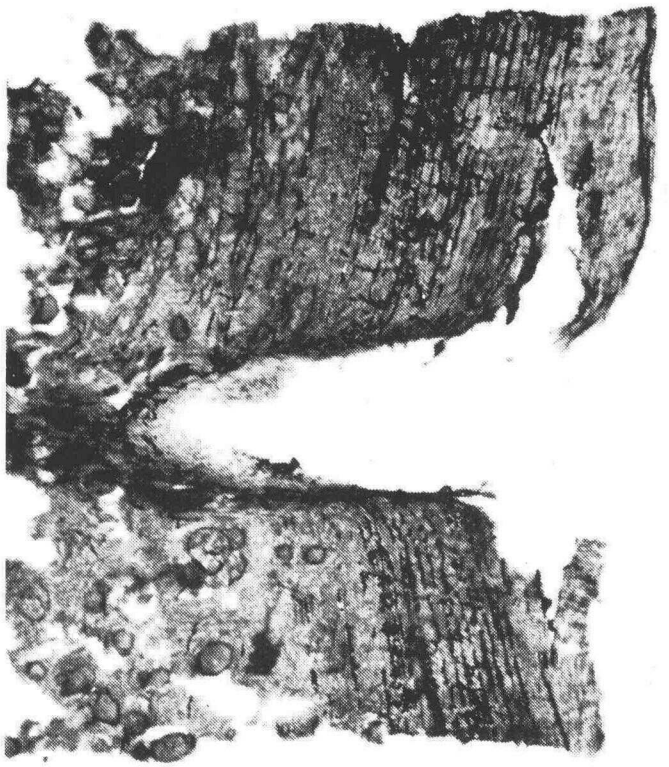


Figure 9. Longitudinal section of RRIM 703 bark showing the cavity developed by wood grown into wound two years after puncture tapping. Magnification 12 X.

CONCLUSION

Taking into account the normal bark reaction to puncture tapping, the damage caused is generally temporary because the normal course of wound healing takes place immediately and new latex vessels are soon regenerated by the undamaged vascular cambium. Latex vessel initiation is at first delayed but probably after two to three years after the original puncture, the renewed bark continues to yield on retapping.

The dark colouration in the scab tissue which disappears when bleached, is due to the presence of substances probably chlorogenic acid which has been shown to be the possible source of chemical units for suberisation during wound healing in sweet potato[18]. On the other hand, the lignified cells observed along the periphery of the 'wounded zone' may indicate the initial development of the hardy tissue into which the whole scab will presumably be converted.

Though the pitted bark presents an unseemly appearance, anatomically it is normal and the regeneration is comparable to that of conventional tapping.

ACKNOWLEDGEMENT

The authors wish to acknowledge the technical assistance of Miss L. H. Ho and Messrs S.M. Yee and F.K. Tsan. Dr Mustaffa bin Ngah is also thanked for some of the provisional translations of foreign papers.

Rubber Research Institute of Malaysia
Kuala Lumpur *January 1981*

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