J. Rubb. Res., **4(3)**, 199–203 **REPRINT**

SHORT COMMUNICATION

Laticifer Initiation and Dense Laticifer Formation in Hevea brasiliensis

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Laticifer initiation and development in Hevea brasiliensis *after bark stripping or wounding was studied. Laticifer cells were not differentiated from the wound-induced callus tissue. The first laticifers formed an irregular row of densely packed cells that resembled the surrounding elongated-parenchyma cells. The subsequent laticifer rings and the laticifer cells therein were similar to those of normal bark. New development of dense laticifers appeared to be induced by the death of mature laticifers or their removal,* e.g. *by tapping.*

Key words: laticifer formation; dense; wound; parenchyma cells; laticifer rings; laticifer cells; bark; tapping; laticifer initiation

Since *Hevea* bark is subjected to regular tapping, a comprehensive knowledge about wound healing, bark regeneration and laticifer formation is very important. Bobilioff[1] briefly described wound healing during normal tapping and bark regeneration when the cambium was injured, but he did not cover laticifer initiation and formation. Wu and Hao[2] observed densely packed laticifer cells around puncture wounds in the bark of tapped rubber trees. Up to now, little is known about this development in *Hevea* bark anatomy. This paper covers some anatomical observations on bark regeneration and laticifer initiation after bark stripping in wounding of *Hevea brasiliensis*. A hypothesis for the formation of dense laticifer cells is proposed.

MATERIALS AND METHODS

Untapped rubber trees (*Hevea brasiliensis*, Muell. Arg., clone RRIM 600), of good vigour that were planted in the Chinese Academy of Tropical Agricultural Sciences in Hainan Island were used for the studies. The experimental treatments were made at a height of 100 cm on the trunks that were about 10 cm – 40 cm in girth. The average ambient temperature in March–September when the experiments were carried out was 27°C, with a maximum of 36°C and a minimum of 23°C.

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Bark Stripping

Fifty trees divided into 10 groups were selected for the treatment. Pieces of bark about 2.5 cm × 2.5 cm were removed together with the cambium from each tree and the wound was covered by wrapping with plastic sheet. The regenerated tissues were sampled and placed in fixative 2, 4, 7, 14, 21, 30, 60 and 120 days after treatment.

Bark Removal to Varying Depths

Ten trees of about 25 cm to 40 cm in girth, were selected for three bark removal treatment as follows[3]:

(1) Removal of the cork layer and a part of the non-conducting phloem without functional laticifers — there was no latex flow;

(2) Removal of all the cork layer and much of the non-conducting phloem with a few functional laticifers — there was only a little latex flow;

(3) Removal of all the tissues outside the conducting phloem and much of the conducting phloem with all the mature laticifers and a part of the immature laticifers — latex flow was considerable.

The above three treatments were carried out at a height of 100 cm high on the different sides of the trunk. All samples were taken two months after treatment.

Bark Wounding by Incision

Five trees were selected for the bark incision experiments. Four blades were inserted at right angles to one another into the bark to the depth of the cambium. This resulted in a square-shaped incision motif. Where the blades inserted into the bark were not removed until 12 h after treatment, little or no latex flowed out. In another treatment where the blades were immediately taken out, latex flow was considerable. Thus, there were two bark incision treatments that resulted either in latex flow or no latex flow.

Sample Processing for Microscopy

Paraffin sections were made from the samples fixed in formalin : acetic acid : 50% alcohol (5:5:90) after appropriate processing. Heidenhain's haematoxylin or fast green was used for staining and I-Br mixed liquid for staining laticifer vessels. The sections (16 μm) were observed by light microscopy.

RESULTS

Bark and Laticifer Regeneration after Bark Stripping

After bark was stripped from the *Hevea* trunk, new bark regeneration involved two stages: callus formation and cambium initiation. Two days after treatment, one of the earliest visible responses was the development of callus from the remaining immature xylem parenchyma cells and xylem ray parenchyma cells adjacent to the surface of the wound. After ten days, the deposition of the wound callus was already up to 1.5 mm in thickness, and the regenerating cambium gradually appeared on the inner portion of the callus. The earliest former phloem elements looked like elongated parenchyma cells ten days after treatment, and were similar to those described by Brown[4]. After one month, the structure of the newly regenrated bark appeared similar to that of normal intact bark.

Laticifers were not differentiated from the wound callus tissue The earliest laticifer cells appeared about three weeks after stripping and were closely packed as an irregular row of laticifers (*Figures 1* and *2*) In longitudinal section, the earliest formed laticifer cells resembled the surrounding elongated-parenchyma cells The cell nuclei were in the centre of the cells and cross-walls between adjoining laticifer cells were evident These features were different from those of normal secondary laticifer cells (*Figure 1*) Unlike the first laticifer ring, the second laticifer ring that developed between one and two months after treatment comprised a single row of laticifer cells The laticifer cells of the second ring were similar to those of normal intact bark (*Figure 2*)

Effects of Bark Removal to Varying Depths

When only the cork layer and a part of the non-conducting phloem was removed, there was no loss of latex Two months after treatment, the first newly formed laticifer ring appeared normal When all the cork layer and the greater part of the non-conducting phloem were removed together with a few functional laticifer cells, there was slight flow of latex Again, the newly formed laticifer ring after treatment was normal In the third treatment where all the tissues peripheral to the conducting phloem (including all the mature laticifer cells and much of the immature laticifers) were removed, copious latex flow ensued Subsequently, most of the remaining immature laticifer tissues died (*Figure 3a*) After two-months, the first newly formed laticifer ring contained 2–4 rows of laticifer cells and was similar to the first laticifer ring in the regenerated bark after stripping In longitudinal section, the features of all the densly packed laticifer cells were the same as for normal secondary laticifers (*Figure 3b*)

Effects of Bark Incision

In the vicinity of the wounding, the bark reacted in the same way as with bark stripping No significant difference in the newly formed laticifer rings was observed between the two incision treatments, with or without latex flow ensuing The newly formed laticifer ring's distant from the wounding were the same as the normal secondary laticifer rings produced before treatment

DISCUSSION

Laticifers were not differentiated from the callus tissue that developed as a response to wounding No laticifer cells could be observed in the regenerating bark until the cambium had regenerated Outside of the first newly formed laticifer ring, there were already some typical sieve elements and accompanying cells that had originated from the regenerated cambium This indicated that the earliest laticifer tissues had originated from the regenerated cambium in the regenerating bark after stripping The earliest formed laticifer cells had some characteristics that were distinct from those of normal secondary laticifer cells The similarity of their shape and structure to the surrounding elongated parenchyma cells, and the presence of cross-walls between adjoining laticifers reflected a primordial stage of laticifer cell differentiation

Wu and Hao[2] first observed that tapped rubber trees could produce many more laticifer rings than the untapped trees, and that densely packed laticifer cells appeared in the vicinity of the wounds after puncturing the bark They opined that latex flow played an important role in enhancing the development of laticifer ring[6] In the present study, it was noted that the dense packing of laticifer

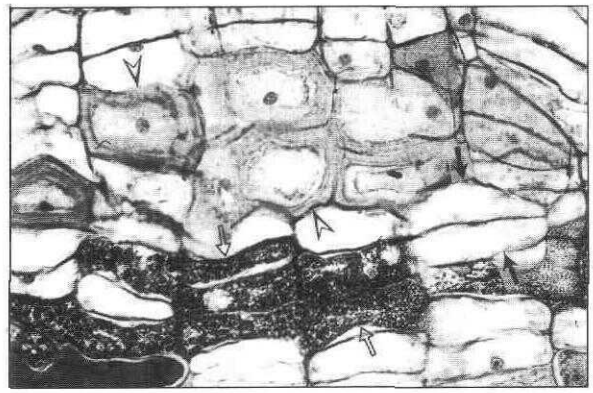


Figure 1. Longitudinal section of regenerated bark 3 weeks after bark stripping, showing densely packed laticifer cells (hollow arrows). These cells resemble the surrounding elongated parenchyma (black arrows). Stone cells (hollow arrow tips) are also visible (× 270).

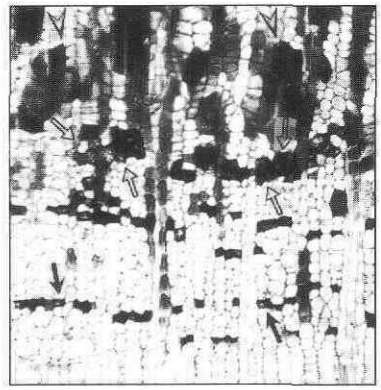
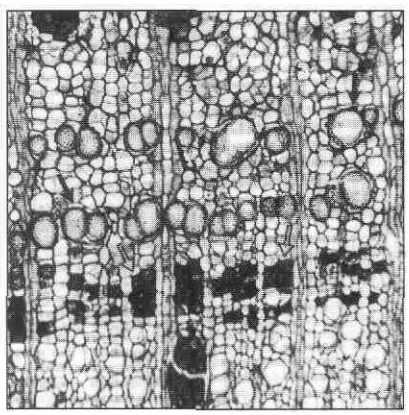


Figure 2. Transverse section of regenerated bark 2 months after stripping, showing the first ring of densely packed laticifer cells (hollow arrows). The newly formed second and third laticifer rings (black arrows) and stone cells (hollow arrow tips) are also seen (× 70).

a



b

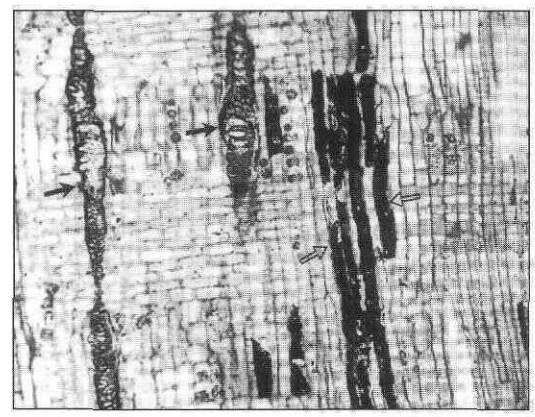


Figure 3. Transverse section (a) and longitudinal section (b) of regenerated bark treated by removing nearly all the functional mature phloem, showing dead and swollen laticifer cells (black arrows). New laticifers that are densely packed and in disorder are seen (hollow arrows). The laticifer vessels are normal (× 70).

cells occurred not only in the regenerated bark after stripping, but also in the vicinity of wounding in general, particularly in the area of the dead swollen laticifer cells around wounds If the dense laticifer formation were attributed to latex flow, it was difficult to explain why the region of closely packed laticifers was far smaller than the latex flow drainage area[7,8] The likelihood that latex flow was not involved was supported by the results of the bark incision experiments where similar responses were encountered whether or not the treatment had resulted in latex loss from the incisions It appeared that mechanical wounding connected within the removal or death of mature laticifer cells resulted in the new formation of the densely packed laticifer cells

Plant tissues and organs can produce inhibitors to inhibit the continued production of the same kind of tissues and organs around them[9] It is hypothesised that this mechanism could occur in *Hevea brasiliensis* and that inhibitors of laticifer development might exist in the mature laticifers Inhibitor activity is lost when laticifers die, leading to an enhancement in laticifer formation in the regenerating bark In the regularly tapped rubber trees, tapping might reduce the concentration of the inhibitors, and this can explain the greater density of laticifers in tapped trees as compared with untapped trees

ACKNOWLEDGEMENT

This research was supported by the National Science Fund of China We thank Tan Haiyan for technical assistance

Date of receipt April 2001
Date of acceptance July 2001

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