J. Rubb. Res., **7(4)**, 281–299

Laticifer Wound Plugging in Hevea brasiliensis*: The Role of a Protein-network with Rubber Particle Aggregations in Stopping Latex Flow and Protecting Wounded Laticifers*

BING-ZHONG HAO*#, JI-LIN WU*, CHUN-XIAO MENG*, ZHENG-QUAN GAO* AND HAI-YAN TAN*

Laticifer wounds were investigated at both the tapping cuts of tapped trees and the bark wounds specially made for investigation. Using light microscopy, a protein-network was found that gradually formed within and over the laticifer wounds as latex flowed. Correspondingly, a network of electron-dense material was observed under electron microscopy. The network, with rubber particle aggregations in its meshes, plugged the laticifer wound when latex flow stopped. The rubber particles of the aggregations retained their intact membrane and did not coagulate until some time after latex flow stopped. Therefore, laticifer wound plugging might not be attributable to the coagulation of the rubber particles but to the formation of a protein-network with rubber particle aggregations. During the course of latex flow after tapping, the activities of chitinase and β-1,3-glucanase, which are known to be the important components among many defense proteins in lutoids, were reduced in expelled latex and increased in the tissue of wounded bark. This indicated that the two enzymes, and perhaps the other defense proteins in lutoids, might be accumulated within and over the laticifer wounds and involved in the composition of the protein-network. Hence the protein-network might function as a biochemical barrier to prevent harmful materials from invading the wounded laticifers. The protective function of the protein-network was demonstrated by experiments in which puncturing and pathogen inoculation were made under the tapping cut and two types of laticifer wound plugs were produced: the normal wound plugs with protein-network and the wound plugs without protein-network formed by in situ *latex coagulation. Under the influence of latex flow, the plugs without a protein-network extended considerably while the normal plugs with protein-network did not. It is consequently suggested that the extension of the laticifer wound plugs without a protein-network might be an essential factor resulting in the onset of the laticifer disease, tapping panel dryness.*

Key words: *Hevea*, laticifer, wounding, defense proteins, tapping panel dryness; latex flow; protein-network; electron microscopy

---

*Key Laboratory of the Ministry of Agriculture for Tropical Crop Physiology, Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan 571737, China

#Corresponding author (e-mail: rcri@public.dzptt.ha.cn)

The latex in laticifers of the rubber tree, *Hevea brasiliensis*, is harvested for manufacturing natural rubber by tapping, or making a cut in the bark to sever laticiferous vessels. As the duration of latex flow after tapping is one of the most important factors limiting latex production, much work has been done on the aspects promoting and hindering latex flow[1–4]. The stopping of flow is generally attributed to the coagulation of latex on the tapping cut and within the laticifer wounds. Lutoids, the vesicles of vacuo-lysosomes, are believed to play a critical role in latex coagulation; damaged lutoids releasing their components during latex flow leading to the coagulation of latex. Several hypotheses have been proposed to explain the mechanisms of latex coagulation[4]. However, most research was made on the experimental systems of latex coagulation *in vitro* and except for that provided by Southorn[5], little knowledge was presented on the laticifer wound plugging in tapped trees.

While it provides commercial rubber for mankind, latex from wounded laticifers might give protection to the wounded bark of the rubber tree itself. The latex might be a type of defense mechanism against pests as resin in pine[6]. When bark is wounded, the sticky latex with its massive flow can hinder the pests from invading the wounds. Moreover, the latex contains various types of molecules capable of preventing pathogens from entering the wounds[7]. Among the molecules are chitinase and β-1,3-glucanase[8–11]. It is proposed that the plugging of the laticifer wounds is concerned with the protective function of latex.

We have found a protein-network in the wounds of the primary laticifers in young shoots of rubber trees[12]. In the present study, we have worked on the secondary laticifers in tapped trees and have showed that a protein-network with rubber particle aggregations has formed during latex flow within the laticiferous vessel ending on the tapping cut. This protein-network may play a fundamental role not only in impeding and stopping latex flow but also in protecting the wounded laticifers and other bark tissue.

MATERIALS AND METHODS

Plant Material

Rubber tree *Hevea brasiliensis* Mull. Arg. buddings of clones RRIM 600, RY-33-97 and Haiken 1 were used in the present study. The trees were grown on the experiment farm of our Academy on Hainan Island, China. The laticifer wounds were investigated at tapping cuts of tapped trees and at the bark wounds specially made for the investigation.

Light Microscopy

Bark samples were fixed in 80% ethanol or FAA (70% ethanol 90 mL, glacial acetic acid 5 mL and formalin 5 mL), and paraffin sections were made by conventional procedures. Mercury-bromophenol blue test was used to detect proteinaceous material in cells, the material staining a clear blue colour[13].

Electron Microscopy

After being excised from tree, bark samples were immediately immersed in chilled 4% glutaraldehyde in 0.1 M phosphate buffer solution at pH 7.2, and then cut into the correct sizes before fixing in the

glutaraldehyde solution at 4ºC for 20 h and fixing in 2% OsO_4 in phosphate buffer for 6 h. The samples were dehydrated in ethanol and embedded in Epon 812 resin. Ultra-thin sections were then cut with an LKB-V microtome, stained in uranyl acetate and lead citrate and examined in a JEM100CX-II electron microscope.

Chitinase Assay

Latex, latex coagulum and bark samples were collected in an ice bath. Latex coagulum and bark were frozen in liquid nitrogen and ground to powder. 1 g sample was suspended in 5 mL 0.01 mol/L sodium acetate buffer (pH 5.0) containing 1% (v/v) Triton X-100®, and centrifuged at 18 000 r.p.m. at 4ºC for 1 h (latex) or 0.5 h (latex coagulum and bark). The supernatant was collected for enzyme assay. Endochitinase was assayed according to Boller and Mauch[14]. The enzyme activity is expressed as nkat/g of fresh sample where nkat is defined as nanomols of N-acetylglucosamine equivalents solubilized per second.

β-1,3-glucanase Assay

Samples were collected as done for chitinase assay and the latex coagulum and bark were also ground to powder. 1 g sample was suspended in 5 mL 0.05 mol/L sodium acetate buffer (pH 5.0), and centrifuged at 18 000 r.p.m. at 4ºC for 1 h (latex) and 0.5 h (latex coagulum and bark). The supernatant was collected for enzyme assay. The enzyme activity was measured by colorimetric method utilising laminarin (Sigma) as a substrate as described by Joosten and De Wit[15]. The enzyme activity is expressed as nmol reduced sugar/s/g of fresh sample.

RESULTS

Protein-Network with Rubber Particle Aggregations Formed within and over Laticifer Wounds during Latex Flow

By using light microscopy, a protein-network was found in and over the laticifer wound of all the tested material, that from different *Hevea* clones with varied ages, with or without tapping and with or without ethephon stimulation. The protein-network of the laticifer wound occurred at the tapping cut or at the bark wound specially made for investigation.

To detect the proteins in laticifers under light microscope, paraffin sections were prepared and stained with mercury-bromophenol blue. The proteins, if they were in large amounts in the laticifers, would be clearly observed in the sections where the rubber of the laticifers had been removed by the solvents n-buty alcohol and xylene used in section preparation. No remarkable proteinaceous material were found in the laticifer vessels in the samples of unwounded bark. In the samples of tapping cut or specially made bark wound, a protein network was observed within and over the ends of the severed vessels (*Figure 1*). The protein network appeared as innumerous fibrils, which inter-weaved with each other and were arranged roughly paralleling the longitudinal axis of the vessel. The network ran down the ends of the severed vessels for a length of about 0.4 m (*Figure 1C*). The protein network was produced gradually during latex flow. We examined the bark samples collected 0, 5, 15 and 30 min after tapping. Immediately after tapping the protein network could not be detected. The network with sparse protein fibrils was visible in the bark samples 5 min after tapping. Along with the duration of latex flow, the protein network became more notable (*Figure 1*).

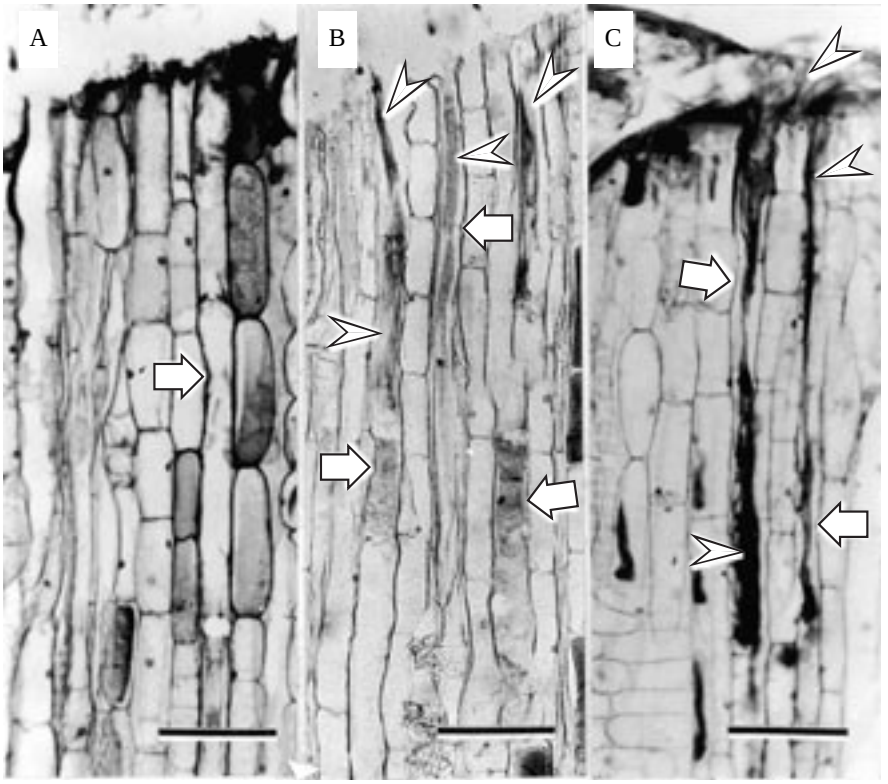

Figure 1. Radial sections of bark at tapping cut showing the formation of protein-network (arrow heads) within and over the laticifer wounds. A: immediately before tapping; B: 5 minutes after tapping; C: 30 minutes after tapping. Arrows: laticifers. Bars = 50 µm.

At electron microscopical level, the laticifer vessel at some distance from the bark wound had intact rubber particles, lutoids and Frey-Wyssling particles (*Figure 2*, where Frey-Wyssling particles are not shown). Rubber particles and scattered election-dense materials but no intact lutoids and Frey-Wyssling particles were found within and over the laticifer wound 1–2 min after tapping (*Figure 3*). The electron-dense materials apparently were pieces of broken lutoids. A network that comprised electron-dense material appeared within and over the laticifer wounds of the wounded bark sampled minutes after latex flow stopped (*Figure 4*). Rubber particles which retained intact membrane were packed in the spaces of the network but the lutoids and Frey-Wyssling particles could not be recognised. By appearance the network in electron micrographs was clearly corresponding with the protein network observed under light microscope.

Under electron microscope, one or two small coagula of a few rubber particles were occasionally found within and over the laticifer

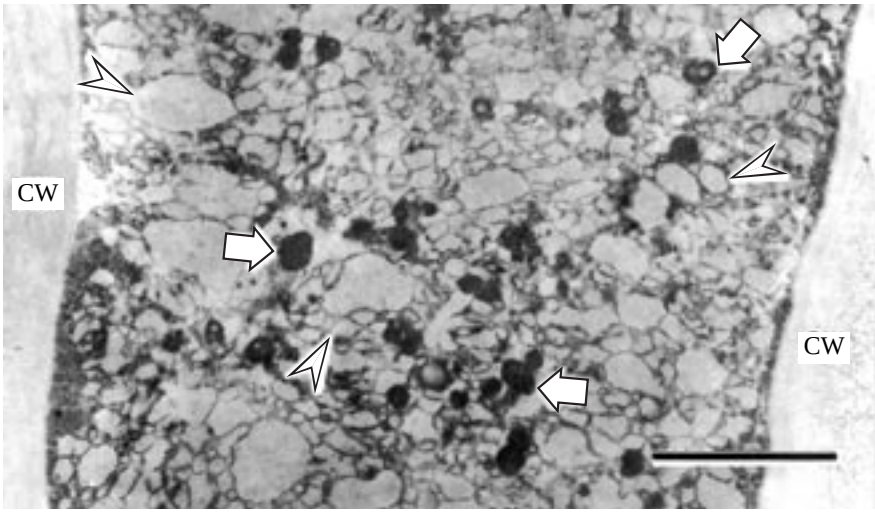

Figure 2. Longitudinal section of laticifer vessel about 2 cm below tapping cut (electron micrograph), sampled immediately before tapping, showing intact rubber particles (arrow heads) and lutoids (arrows). CW: cell wall. Bar = 5 µm.

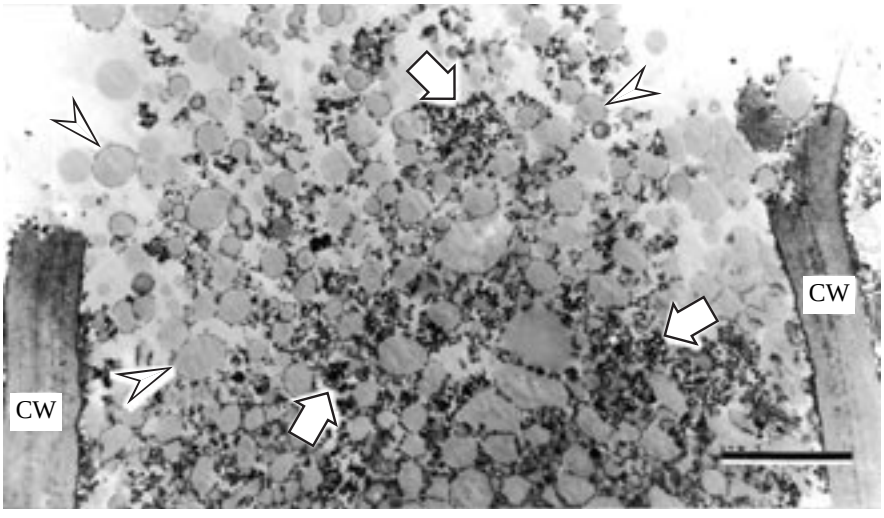

Figure 3. Longitudinal section of laticifer vessel wound about 1 min after tapping (electron micrograph) showing rubber particles (arrow heads) and electron-dense materials (arrows). Bar = 5 µm.

Figure 4. Longitudinal section of laticifer vessel wound about 1 min after flow stopped (electron micrograph) showing the network formed from electron-dense materials (arrows) and the rubber particle aggregations (arrow heads). CW: cell wall. Bar = 5 µm.

wounds before latex flow stopped. Immediately after the flow had stopped, a film formed from coagulated rubber particles was seen on the surface of the latex over the tapping cut (*Figure 5*). However, the rubber particles trapped in the network of electron dense material did not coagulate until some time after the latex flow had stopped: for instance, the coagula of the rubber particles were found in the laticifer wound 24 h after the latex flow had stopped (*Figure 6*).

No Protein-network Arose in Laticifer Wounds without Latex Flow

An obvious protein-network was never found by light microscopy in and over the laticifer wounds where no or a little latex flowed out. Here the laticifer wounds were finally plugged by *in situ* coagulated latex. This appeared in many conditions, some of which will be described below (see *Figures 9*, *11* and *12*). When the tree bark was punctured with a needle that was kept in the bark to minimise exudation of latex at the puncture, *in situ* coagulation of latex in the laticifer wounds around the puncture finally occurred about 24 h after puncturing and resulted in plugging of the laticifer wound. These plugs therefore had no obvious protein-network. (*Figure 7*).

During the Course of Latex Flow after Bark Wounding, Activities of Chitinase and β-1,3-glucanase were Reduced in Expelled Latex and Increased in the Tissues of the Bark Wound

Chitinase and β-1,3-glucanase activities were measured in the expelled latex collected successively after tapping. This was done in *Hevea* clones Haiken 1 and RRIM 600 (12 years old, untapped before the experiment), and gave similar results, and the ones from RRIM 600 are shown in *Figure 8*. The highest activities of the two enzymes was in the initial latex during latex flow. During the course of flow, the enzyme activities were reduced. The initial latex of a new tapping below the previous tapping cut resumed the high level of the enzyme activities. These results indicated that during latex flow a large amount of the enzymes in latex may be obstructed in the laticifer wounds.

To test the chitinase and β-1,3-glucanase activity in bark wound tissue, bark wounding experiments were done as shown in *Figure 9*, in which two types of bark wounds were produced: bark wounds with normal latex flow and bark wounds with limited latex flow. The experiment was repeated three times in *Hevea* clone Haiken 1 and RRIM 600 (12 years old, untapped before the experiment) and gave similar results, (*Figure 10*). In the bark tissue from the wound with normal latex flow, the activities of the two enzymes increased with the course of latex flow after wounding. No obvious change in the enzyme activities was observed in the tissue either from the wound with limited latex flow or from the unwounded bark (the bark 2 cm below the bark wound with normal latex flow). Therefore the increase of the enzyme activities in wounded bark tissues with latex flow must have been caused by the flow and a deduction can be made that the increased enzyme activities resulted from the accumulation in the laticifer wounds of the enzymes from latex, during latex flow. This deduction obtains support from the above experiment (*Figure 8*) which showed a gradual reduction in the activities of the two enzymes in the latex collected successively after tapping. Moreover as plugs with protein-network formed in the laticifer wounds with latex flow, the two enzymes accumulated in the wounds might be involved in constructing the protein-network.

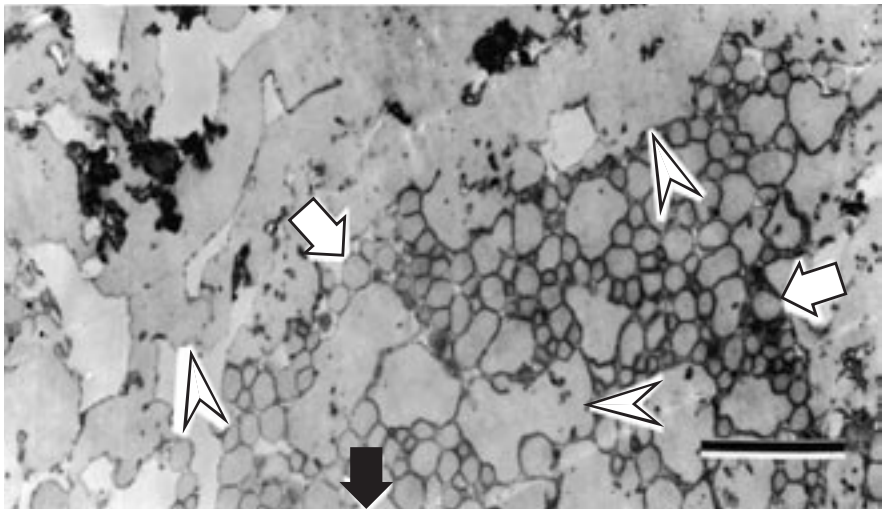

Figure 5. Section through the latex over tapping cut about 1 min after latex flow stopped (electron micrograph) showing the coagula (arrow heads) of rubber particles. White arrows: rubber particles. Black arrow is directed to tapping cut. Bar = 3 µm

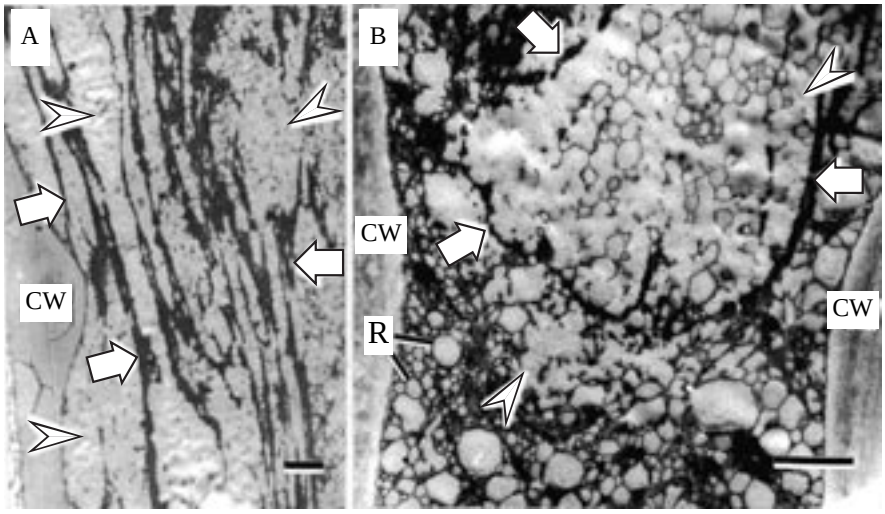

Figure 6. Longitudinal section of laticifer vessel wound 24 h after latex flow stopped (electron micrograph) showing the coagulated rubber particles (arrow heads) and the network of electron-dense material (arrows). A: near the wound end; B: further from the wound end; R: rubber particles. Bar = 3 µm

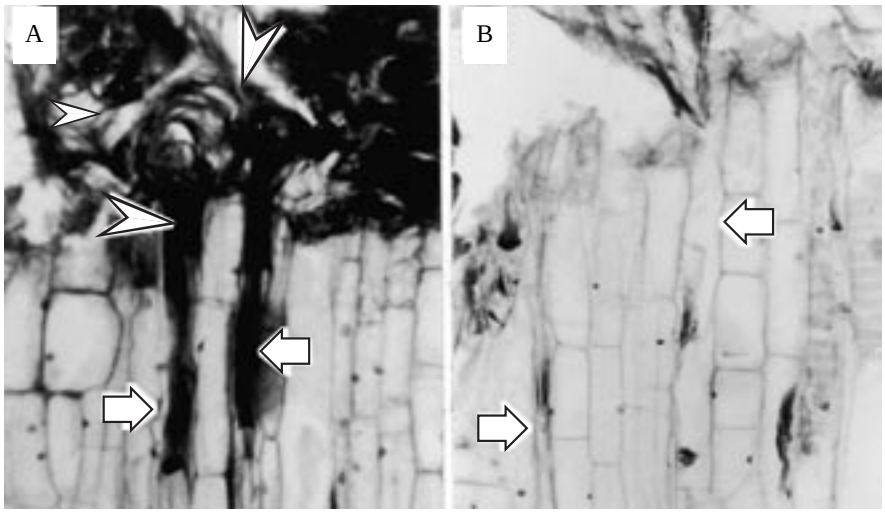

Figure 7. Radial section of bark through the punctures showing the laticifer wounds with obvious protein-network (arrow heads) in the puncture with normal latex flow (A) and laticifer wounds without obvious protein-network in the puncture without latex flow (B).

The Wounded Laticifer Having the Wound Plug without Protein-Network was Liable to be Affected by Latex Exploitation and Exogenous materials

To test the effect of latex exploitation on the existing laticifer wound plugging, the bark puncturing experiment was done as shown in *Figure 11*, in which two types of laticifer wounds were produced: the wounds which underwent latex flow and formed the protein-network, and those without latex flow and without the protein-network. Under the influence of latex exploitation, the laticifer wound plug without the protein-network extended along the direction of the latex flow while no significant extension took place for the plugs with the protein-network.

An inoculation experiment (*Figure 12*) was done with *Phytophthora citrophora*, that is the pathogen causing black stripe in tree bark. The disease lesion with extension potential developed more greatly under the influence of latex flow than that without the influence. Although it is general knowledge that tapping causes the extension of the unstable lesion, we draw attention to the fact that the lesion extended along the direction of latex flow and hence along the laticifer wounds which did not undergo latex flow and had no protein-network.

DISCUSSION

Normal Laticifer Wound Plugging is not Attributed to the Coagulation of Rubber Particles but to the Formation of Protein-network with Rubber Particle Aggregations

In the present study, we discovered that a protein-network with rubber particle aggregations gradually forms within and over the

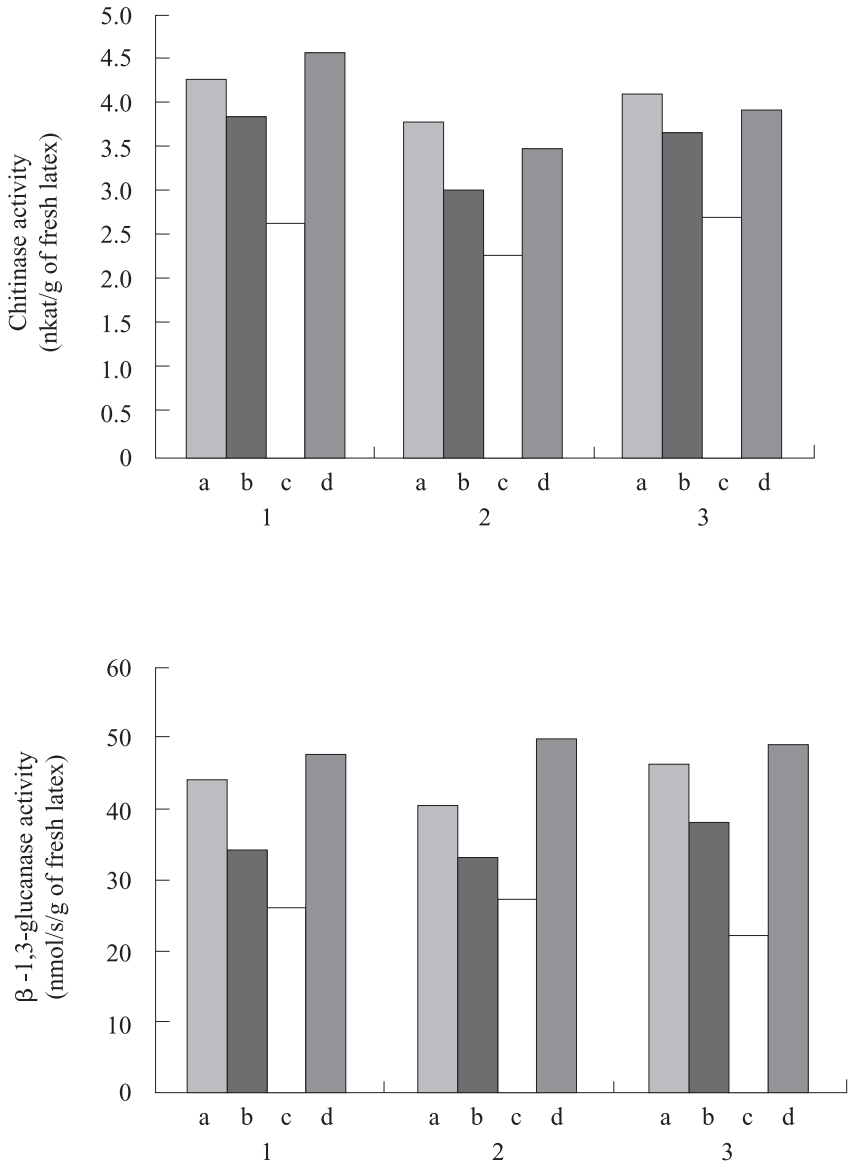

*Figure 8. Chitinase (A) and β -1,3-glucanase (B) activities in latex collected successively after tapping (*Hevea *clone RRIM 600). The times when the latex samples were collected: a, immediately after tapping; b, 15 min after tapping; c, approaching stop of latex flow (about 50 min after tapping); d, immediately after a new tapping that was done after stoppage of the previous flow. The enzyme assays were made in three trees (1–3).*

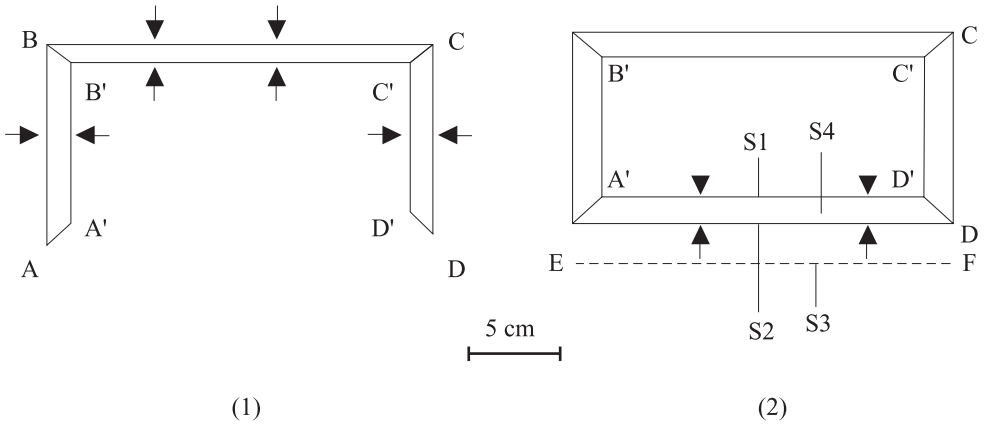

Figure 9. Schematic diagram of bark wounding experiment: (1) Bark was cut into wood to remove bark stripe AA'B'B, BB'C'C and CC'D'D. Latex flowed out from the bark wounds (arrows). (2) A few minutes after the flow stopped, a new wound AA'D'D was made. Normal latex flow (arrows) occurred from AD, but a little latex flowed out (arrow head) from A'D' since the latex had a limited source, the laticifers in A'B'C'D'. Samples for enzyme assay: bark wounded with limited latex flow (S1); bark wounded with normal latex flow (S2); bark (EF) below wound with normal latex flow (S3); and latex over bark wound AA'D'D (S4). Bark samples were collected by cutting bark 1 mm thick along the wounds and the inner bark was used for enzyme assay.

laticiferous vessel ends during latex flow when the vessels are wounded, for instance, by tapping. The development of the protein-network coincides with decrease of latex flow rate and the protein-network represents a prominent part of the contents in laticifer wounds during the stopping of latex flow. We therefore believe that the protein-network plays an important role in impeding and stopping latex flow from laticifer wounds.

Our observations are substantially different from the work made by Southorn[5], which was the only previous study on the tapping cut of the rubber tree. According to Southorn, the obstruction to and the final stopping of latex flow resulted from the coagulum of rubber particles that formed the coagulum cap over the tapping cut and coagulum plugs within the laticiferous vessels. The plugs abruptly formed and completely sealed the individual vessels soon after tapping and they usually occurred without any obvious connection with the cap.

Thus two differences can be found between Southorn's conclusion and our observations. Firstly, in our study, no coagulum with significance but aggregations of rubber particles were found trapped in the protein-network within and over the laticifer wounds when latex flow stopped. The aggregations of rubber particles did not coagulate until some time after latex flow stopped. Here, the 'aggregations' indicate the bodies consisting of rubber particles that retain their intact membranes and have no contact between the rubber phase of the different particles

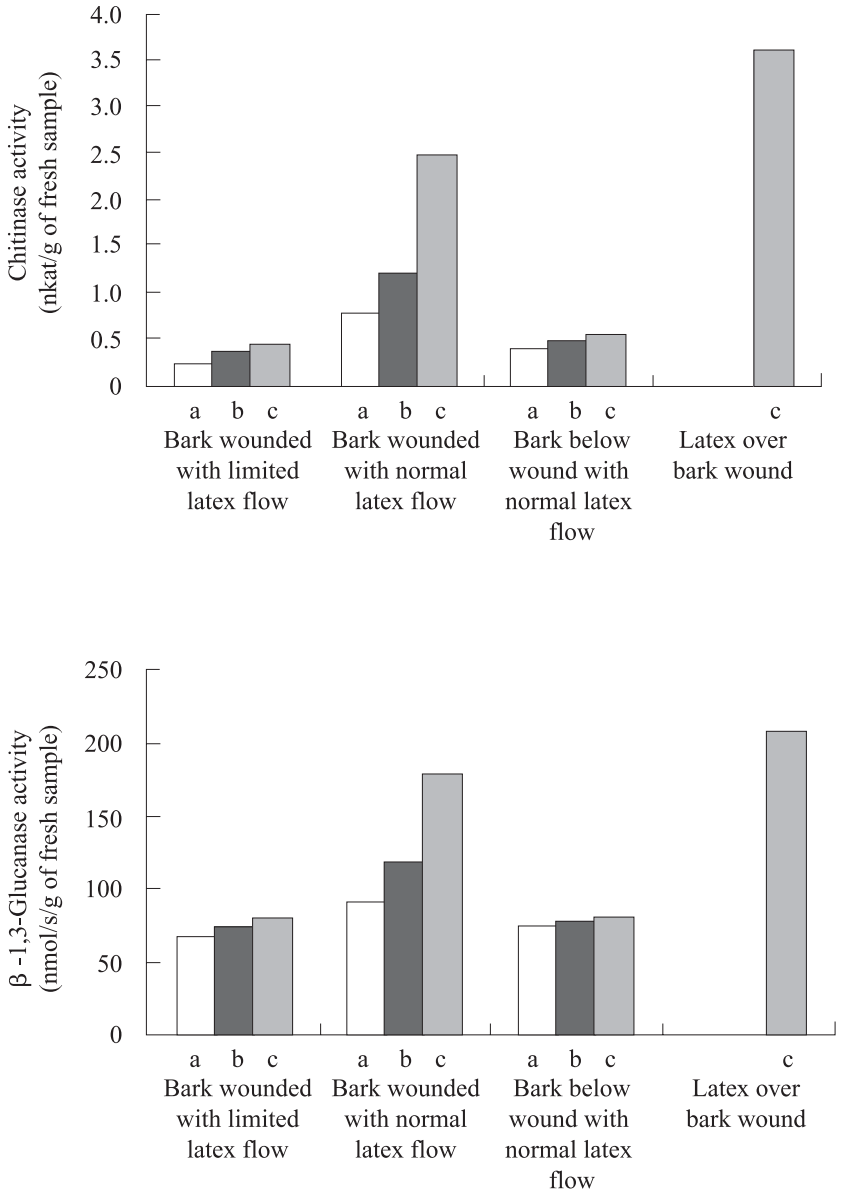

*Figure 10. Chitinase (A) and β -1,3-glucanase (B) activities in bark wound tissue and in latex over the bark wound (*Hevea *clone RRIM 600). The bark wounding experiment is illustrated in* Figure 9. *The bark was frozen by liquid nitrogen immediately after bark samples were collected to protect the bark from latex loss. The times when the samples were collected: a, immediately after wounding; b, 10 min after wounding; c, a few minutes after latex flow stopped (about 40 min after wounding). Each enzyme activity value is the mean of the values from three trees.*

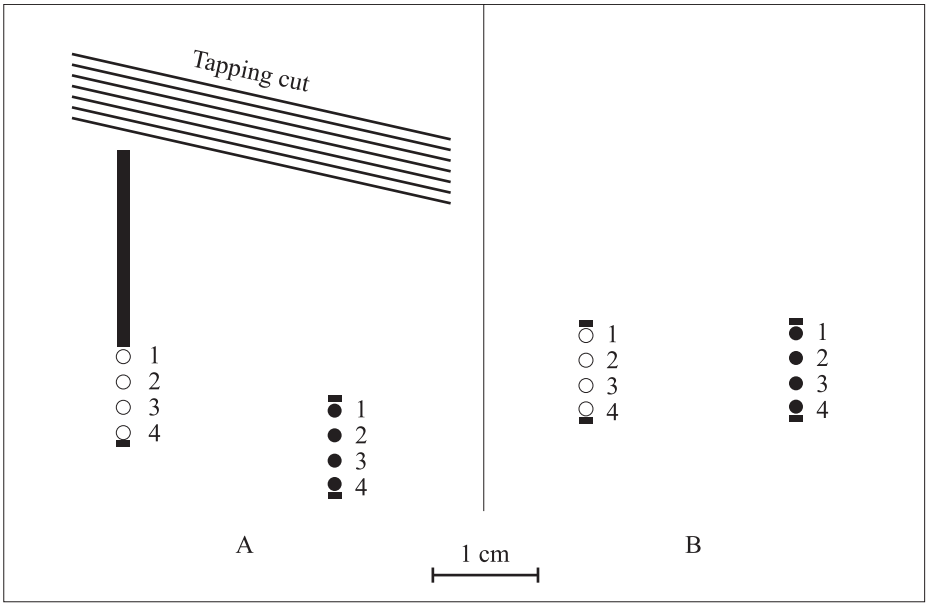

*Figure 11. Bark puncturing experiment showing the extension of the laticifer wound plugs without protein-network under the influence of latex exploitation (*Hevea *clone RY 7-33-97, 3 years old). A: Puncturing with tapping. Bark punctures (1–4) were made with pins (nickel plated) of 0.8 mm diameter. ●: Punctures with normal latex flow and consequently with protein-network in the laticifer wound plugs. ○: Punctures with the pins remaining in the punctures for 18 h, having no latex flow and no protein-network in the laticifer wound plugs. Tapping was done 18 h after a puncture was made, and in this way four tappings and four punctures (1–4) were made. Another tapping without puncturing was made each day for three days. 8 days after puncturing, the scope of the laticifer wound plugs was measured by puncturing the bark, no or little latex flow indicated the existence of the plugs. Bars above and below the punctures indicate the plugscope in longitudinal direction and the bar lengths are mean values of five samples. B: The punctures (1–4) were made as was done in A but without tapping.*

while the 'coagulum' points to what is formed by fusion between the rubber phase of the rubber particles[16]. Since the rubber particle aggregations are trapped in the protein-network, we deduce that it would be difficult for the aggregations without a protein-network to seal the laticifer wounds and stop latex flow. Thus the protein-network might be more important than the rubber particle aggregations for the laticifer wound plugging. Secondly, our observations showed that the laticifer wound plugging occurred gradually during latex flow so that within and over the laticifer wounds a considerable amount of proteins could have been accumulated that constituted the protein-network, in which defense proteins from lutoids could have been included (see below). In contrast, according to Southorn[5], as the laticifer wound plugs formed abruptly, no proteins in quantities could have been accumulated within and over the laticifer wounds.

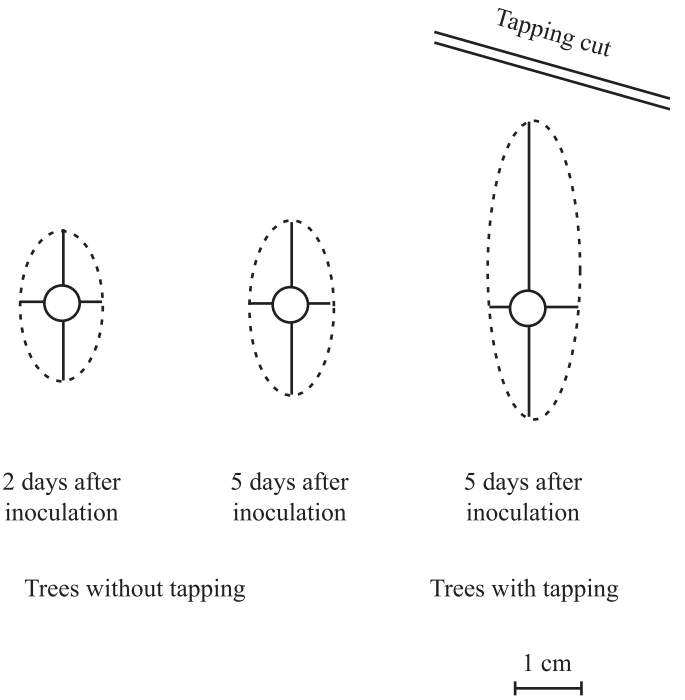

Figure 12. Inoculation experiment with a fungus Phytophthora citrophora *RbCH (*Hevea *clone RY7-33-97, 4-year-old). The fungus in agar was inoculated in the bark wound (O), and kept wet for 1 day, and 2 days after inoculation the trees were tapped each day for two days. The infection scope was measured by puncturing the bark; no latex flow indicated the presence of tissue necrosis. The measurements (bars around the bark wounds) are mean of 5 samples.*

So far it has been generally accepted that the obstruction and stoppage of latex flow after tapping is attributed to the formation of coagula from rubber particles and this process is the same in principle as that occurring *in vitro*. However, our studies show that the laticifer wound plugging might involve very complex processes, only one of which was the formation of the rubber particle aggregations. Therefore, normal laticifer wound plugging might not be completely explained on the present hypothesis that were proposed to elucidate the mechanisms leading to the coagulation of rubber particles and were mainly based on the experiments of latex coagulation *in vitro*[3–4].

Protein-network May be a Biochemical Barrier to Prevent Harmful Substances from Entering the Wounded Laticifer Vessels

Available evidence goes to show that most of the composition of the protein-network might have an origin from lutoids. It has long been known that during latex flow after tapping, damaged lutoids are present within laticifer wounds and on the tapping cut[1]. This is also

shown in *Figure 3*. More significantly, Pakianathan and Milford[17] demonstrated that during latex flow a considerable accumulation of the damaged lutoids occurs most likely within and over the cut ends of laticiferous wounds. These facts indicate a possibility that the proteins released by the damaged lutoids are involved in the composition of the protein-network.

Our study gives more direct evidence for the origination of the protein-network. By determination of the activities of the chitinase and β-1,3-glucanase in latex and bark tissues during latex flow, we have shown that a great amount of the two enzymes from latex have accumulated within and over the laticifer wounds. The two enzymes, expected to come from lutoids as chitinase and β-1,3-glucanase are known to be the important components among many defense proteins in lutoids[8–11]. We therefore suggest that the two enzymes, and perhaps the other defense proteins in lutoids, are involved in the composition of the protein-network.

The involvement of defense proteins in the composition of the protein-network indicate that the protein-network may function as a biochemical barrier to prevent harmful substances from invading the intact parts of wounded laticifers when latex flow has stopped. This is clearly demonstrated by the experiments in which bark puncturing (*Figure 11*) and pathogen inoculation (*Figure 12*) were made below the tapping cut. These experiments showed that the laticifer wound plugs without protein-network extended along the laticifer vessels when latex exploitation was made from the tapping cut, while the wound plugs with protein-network did not get extended. A reasonable explanation for these facts is that under the influence of latex movement caused by exploitation, some harmful substances go into the intact parts of the laticifers through the wound plugs without a protein-network and lead to latex coagulation, while the plugs with protein-network are effective to prevent the harmful substances from entering the intact parts of the laticifers. Here the harmful materials might be those that came from extra-laticifers or were formed in wounded parts of the laticifer vessels. We therefore designate the wound plug without protein-network as imperfect plug, and the wound plug with protein-network as perfect plug. Correspondingly plugging with the imperfect plug is described as imperfect and plugging with perfect plug as perfect.

Imperfect Plugging of Laticifer Wounds Might be A Fundamental Mechanism Leading to Tapping Panel Dryness

One of the most important diseases of rubber tree is a laticifer disease called tapping panel dryness (TPD) or bark dryness[4,18–21]. For a TPD symptom, it is essential that *in situ* coagulation of latex develops in laticifers of the area below the tapping cut and results in abnormally low or stoppage of latex production.

The protective function of the protein-network implicates it in the onset of TPD. As shown in the experiment of bark puncturing (*Figure 11*), under the influence of latex exploitation, *in situ* latex coagulation developed from the imperfect plugs of the laticifer wounds below tapping cut. It can be expected that considerable development of this process will finally lead to TPD. We therefore propose that the development of *in situ* coagulation of latex from the laticifer wounds with imperfect plugs is a fundamental mechanism leading to TPD. In agreement with this, Sivakumaran and Pakianathan[22] developed

a rapid method of inducing TPD in a rubber tree. The method involves puncturing the bark below the tapping cut and sealing the punctures to minimise the amount of latex exudation. Just based upon this, we developed the method used for our bark puncturing experiment, therefore induced TPD would result from the same mechanism as revealed in our experiment.

TPD can be induced by varied factors[18]. In most conditions the onset of TPD might be related to the imperfect plugging of laticifer

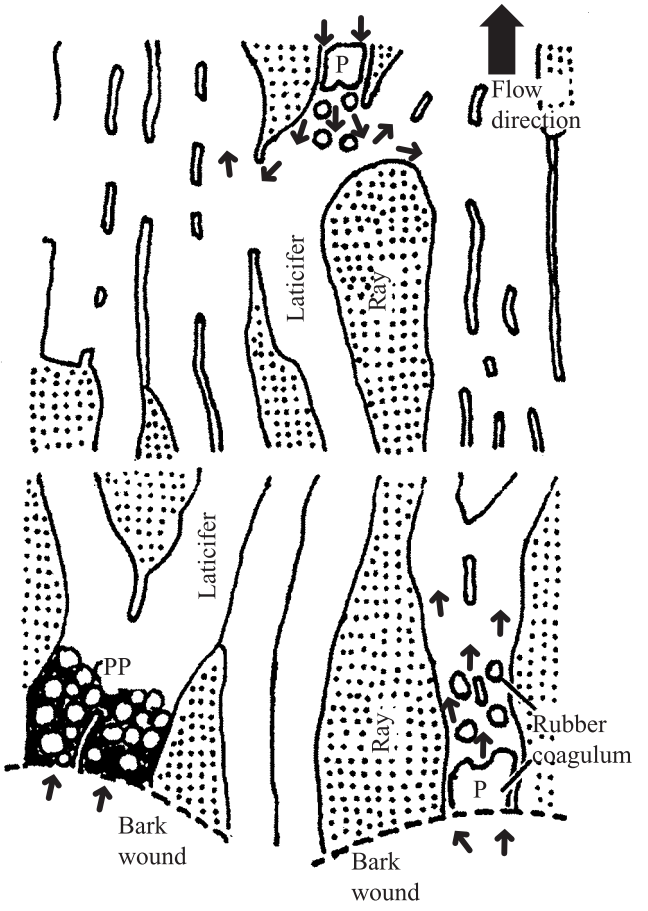

Figure 13. A putative model for the protective function of the protein-network in laticifer wounds as well as the onset of TPD. Tangential section of laticifer ring cross tapping cuts. In the laticifer wound plugs without protein-network (P), the material (small arrow) out of laticifer or formed in the wounded parts of the laticifer could diffuse to its intact parts under the influence of latex flow. Diffusion of the material would be prevented in the laticifer wound plugs with protein-network (PP).

wounds. For example, in over-exploited trees a higher incidence of TPD is caused by destabilization of latex and subsequent *in situ* coagulation of latex in laticifers near the tapping cut[19]. We suggest that without a protein-network the latex coagula would form imperfect plugs in the laticifer vessels or at the ends of the wounded vessels. The coagula therefore cannot prevent the materials harmful to latex from spreading under the influence of tapping and this would result in the onset of TPD. The other example of TPD is when the bark wound is kept from healing in roots, and TPD originated from the wound, and spread rapidly upwards to the tapping cut[23]. In this case the origination of TPD would also be concerned with the imperfect plugging of laticifer wounds since the imperfect plugging of laticifer wounds, would be expected to occur in the bark wounds kept from healing, as in the case of the experiment of pathogen inoculation (*Figure 12*). Finally, a putative model for the protective function of the protein-network at laticifer wounds as well as the onset of TPD is made (*Figure 13*) where three factors would be essential to the induction of TPD: the imperfect plugging of laticifer wounds; the influence of latex flow; and the presence of the material that are harmful to the laticifers.

As D'Auzac[3] pointed out, the stopping of latex flow at the tapping cut, and the latex coagulation *in vitro* (in the latex cup or in the factory) do not necessarily involve the same phenomena, or at least to the same extent. However, there has been only little research dealing with this subject except for the study on the role of bark cell sap in latex coagulation made by Gomez *et al*. (see review[3]). The discovery of the protein-network gave further emphasis to the significant difference in the latex behaviour around laticifer wounds and out of the tree. This discovery might greatly facilitate our understanding of the mechanisms of the laticifer wound plugging although the protein-network is now only slightly known. Further studies are needed to obtain more information about the composition, formation and function of the protein-network. A recent research showed that the actin cytoskeleton in laticiferous cells may influence the important role in the formation of protein-networks[24].

ACKNOWLEDGMENT

This study was supported in the early stage by the Science Foundation of China (No. 39160046). We thank Zhou Hui-Lin for help in electron microscopy, and Li Rui for providing the inoculum of *Phytophthora citrophora*.

Date of receipt: June 2004
Date of acceptance: October 2004

REFERENCES

1. SOUTHORN, W.A. (1969) Physiology of *Hevea* (Latex Flow) *J. Rubb. Res. Inst. Malaya*, **21**, 495–512.

2. GOMEZ, J.B. (1983) *Physiology of Latex (Rubber) Production*. MRRDB Monograph No. 8, pp. 115.

3. D'AUZAC, J. (1989) Factors Involved in the Stopping of Flow after Tapping, *Physiology of Rubber Tree Latex (D'Auzac, J., Jacob, J.L. and Chrestin, H. eds.)*, Chap. 4.3, pp. 257–280. Boca Roton: CRC Press.

4. D'AUZAC, J., JACOB, J.L. PRÉVÔT, J.C., CLEMENT, A., GALLOIS, R., CHRESTIN, H., LACOTE, R., PUJADE-RENAUD, V., MOWTORO, P. AND GOHET, E. (1997) The Regulation of *Cis*-Polyisoprene Production (Natural Rubber) from *Hevea brasiliensis*. *Recent Res. Plant Physiol*., **1**, 273–331.

5. SOUTHORN, W.A. (1968) Latex Flow Studies 1. Electron Microscopy of *Hevea brasiliensis* in the Region of the Tapping Cut. *J. Rubb. Res. Inst, Malaya*, **20**, 176–186.

6. NAGY, N.E., FRANCESCHI, V.R., SOLHEIM, H., KREKLING, T. AND CHRISTIANSENE (2000) Wound-induced Traumatic Resin Duct Development in Stems of Norway Spruce (Pinaceae): Anatomy and Cytochemical Traits. *Amer. J. Bot.*, **87**, 302–313.

7. D'AUZAC, J., PRÉVÔT, J.-C. AND JACOB, J.-L. (1995) What's New about Luloids? A Vacuolar System Model from *Hevea* Latex. Plant Physiol. Biochem., **33**, 765–777.

8. MARTIN, M.N. (1991) The Latex of *Hevea brasiliensis* Contains High Levels of Both Chitinases and Chitinases/Lysozymes. *Plant Physiol*., **95**, 469–476.

9. BRETON, F., COUPÉ, M., SANIER, C. AND D'AUZAC, J. (1995) Demonstration of β-1,3-Glucanase Activities in Lutoids of *Hevea brasiliensis* Latex. *J. nat. Rubb. Res*., **10**, 37–45.

10. CHURNGCHOW, H., SUNTARO, A. AND WITITSUWANNNAKUL, R. (1995) β-1,3-Glucanase Isozymes from the Latex of *Hevea brasiliensis*. *Phytochemistry*, **39**, 505–509.

11. SUBROTO, T., VAN KONINGSVELD, G.A., SCHREUDER, H.A., SOEDJANAATMA U.M.S. AND BEINTEMA, J.J. (1996) Chitinase and β-1,3-Glucanase in the Lutoid-body Fraction of *Hevea* latex. *Phytochemistry*, **43**, 29–37.

12. HAO, B., WU, J. AND TAN, H. (1996) Laticifer Plugging of *Hevea brasiliensis* after Severing. *Chinese J. Trop. Crops*, **17(1)**, 1–6.

13. PEARSE, A.G.E. (1960) *Histochemistry, Theoretical and Applied*. London: J. and A. Churchill Ltd. pp. 792.

14. BOLLER, T. AND MAUCH, F. (1988) Colorimetric Assay for Chitinase. *Methods Enzymal*., **161**, 430–435.

15. JOOSTEN, M.H.A.J. AND DE WIT, P.J.G.M. (1989) Identification of Several Pathogenesis-related Proteins in Tomato Leaves Inoculated with *Cladosporium fulvum* (syn. *Fulvia fulva*) as β-1,3-Glucanase and Chitinases. *Plant Physiol*. **89**, 945–951.

16. COCKBAIN, E.G. AND PHILPOTT, M.W. (1963) Colloidal Properties of Latex, *The Chemistry and Physics of Rubber-like Substances (Batman, L. ed.)* Chap. 4, pp. 73–95. London: Maclaren and Sons Ltd.

17. PAKIANATHAN, S.W. AND MILFORD, G.F.J. (1973) Changes in the Bottom Fraction Contents of Latex during Flow in *Hevea brasiliensis. J. Rubb. Res. Inst. Malaya*, **23**, 391–400.

18. DE FAŸ, E. AND JACOB, J.-L. (1989) Symptomatology, Histological, and Cytological Aspects, *Physiology of Rubber Tree Latex (D'Auzac, J., Jacob, J. L. and Chrestin, H. eds.)*, Chap. 7.1, pp. 407–430. Florida: CRC Press.

19. CHRESTIN, H. (1989) Biochemical Aspect of Bark Dryness Induced by Over Stimulation of Rubber Trees with Ethrel, *Physiology of Rubber Tree Latex (D'Auzac, J., Jacob, J. L. and Chrestin, H. eds.)*, Chap. 7.2, pp. 431–441. Florida: CRC Press.

20. ESCHBACH, J.-M., LACROTTE, R. AND SERRES, E. (1989) Conditions which Favor the Onset of Brown Bast, *Physiology of Rubber Tree Latex (D'Auzac, J., Jacob, J. L. and Chrestin, H. eds.)*, Chap. 7.3, pp. 443–458. Florida: CRC Press.

21. PARANJOTHY, K., GOMEZ, J.B. AND YEANG, H.Y. (1976) Physiological Aspects of Brown Bast Development. *Proc. Int. Rubb. Conf. 1975, Kuala Lumpur*, **2**, 181–202.

22. SIVAKUMARAN, S. AND PAKIANATHAN, S.W. (1983) Studies on Tree Dryness. 1. A Simple and Rapid Method of Inducing Dryness in *Hevea* Trees. *J. Rubber Res. Inst. Malaysia*, **37**, 88–101.

23. WU, J.-L., TAN, H.-Y., TIAN, W.-M. AND HAO, B.-Z. (1997) Tapping Panel Dryness Syndrome in *Hevea brasiliensis* Associated with Wounds in the Root. *Indian J. nat. Rubb. Res*., **10**, 102–106.

24. GAO, Z., MENG, C., WU, J. AND HAO, B. (2003) Actin Cytoskeleton in Laticiferous Cells of *Hevea brasiliensis* in Relation to Latex Exploitation. *Chinese J. Trop. Crops*, **24(3)**, 22–26.