J Wood Sci (2008) 54:414–419
DOI 10.1007/s10086-008-0959-3

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Erwin · Won-Joung Hwang · Yuji Imamura

Micromorphology of abnormal and decayed xylem in rubberwood canker

Received: October 19, 2007 / Accepted: February 15, 2008 / Published online: July 26, 2008

Abstract Typical symptoms of rubberwood canker found in East Kalimantan, Indonesia, differ from those of conventional rubberwood peach canker. Rubberwood canker showed a conspicuous longitudinal canker with exposed xylem that was discolored and decayed. Bark wounds, resulting from regular tapping, were enclosed within the xylem, and the tissues had become integrated. The characteristics of abnormal and decayed xylem in this rubberwood canker were analyzed by light microscopy and scanning electron microscopy. Xylem in the vicinity of the enclosed bark contained fewer vessels of smaller diameter and shorter length, and significantly wider rays compared with normal xylem. Around the wide growth zones of the canker, axial cells were disoriented and warped toward the canker zones. In view of the separation among cells, and the concentric degradation of the cell walls starting from the lumen surfaces, decayed xylem appeared to be caused mainly by white-rot fungal attack.

Key words Canker · Rubberwood · Decayed xylem · Enclosed bark · Wood anatomy

Introduction

In the past 30 years, rubber production has played an important role in the economy of Indonesia. Today, Indonesia is the world's second-largest producer of natural and semi-processed rubber after Thailand. Rubber trees (*Hevea brasiliensis*, Muell. Arg) in plantations are generally felled for replanting after 25 to 30 years, due to reduced latex production. The timber is used to make furniture and other wood-based products.

Recently, a canker disease afflicting the rubber tree has been discovered in a plantation in East Kalimantan, Indonesia. The disease showed symptoms similar to those of a stem canker disease called "peach canker." This disease is common in rubber trees,[1] showing discolored phloem tissue and exposed xylem tissue as well as cracks and ruptures on the surfaces of the bark near to the canker zone. The newly discovered canker, however, consisted of discolored and decayed tissue, with the exposed xylem occurring longitudinally along the side of the stem, causing the tree shape to be deformed. Furthermore, the bark wound caused by regular tapping was completely closed by callus formation, which in turn was enclosed within the xylem tissue. The bark enclosed by xylem had formed in circles along the parts of the stem that had been tapped repeatedly.

Until now, the decayed xylem of rubberwood canker and the features of the xylem tissue enclosing the bark wound caused by regular tapping have not been studied. As a first step to establish the characterization of rubberwood canker and to elucidate its development, we used light microscopy and scanning electron microscopy techniques to observe the microscopic features of the xylem in the vicinity of the enclosed bark and the decayed xylem in the canker margin.

Materials and methods

Sample collection

Five disks of the canker stem (approximately 5 cm in thickness) were obtained from a 30-year-old rubber tree [diameter at breast height (DBH) 55 cm] growing in the plantation in East Kalimantan, Indonesia. The canker occurred in the stem near the ground. The length and width of the canker with exposed xylem were 50 cm and 24 cm, respectively

Erwin (✉) · Y. Imamura
Research Institute for Sustainable Humanosphere (RISH), Kyoto University, Gokasho, Uji 611-0011, Japan
Tel. +81-774-38-3663; Fax +81-774-38-3664
e-mail: erwin@rish.kyoto-u.ac.jp

W.-J. Hwang
Institute of Wood Technology, Akita Prefectural University, Noshiro 016-0876, Japan

Fig. 1. A longitudinal canker with exposed xylem of rubberwood (*arrow*)

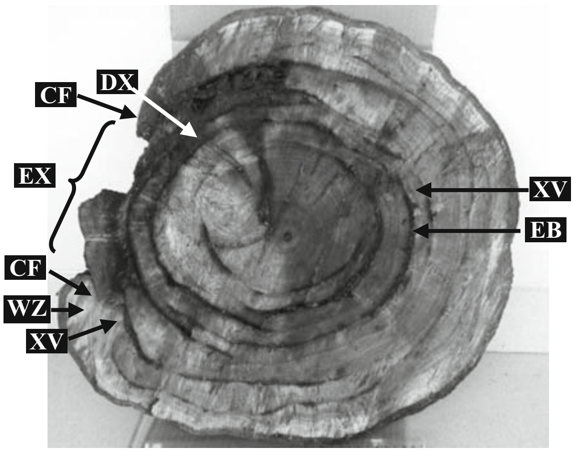

Fig. 2. Transverse view of the canker disk with exposed xylem (*EX*), decayed xylem (*DX*), wounded bark enclosed within xylem (*EB*), xylem in the vicinity of the enclosed bark (*XV*), callus formation (*CF*), and wide growth zone (*WZ*)

(Figs. 1 and 2). For comparison, disks of normal stem were obtained from a healthy tree standing close by.

Light microscopy observations

The xylem samples (approximately 15 × 15 × 15 mm) taken from the margins of the decayed xylem (DX), xylem in the vicinity of the enclosed bark on the opposite side and adjacent to the canker zone (XV), and normal xylem were fixed with formalin/acetic acid/50% ethanol (FAA). After washing under running water for 2–4 h, they were dehydrated in an ethanol series and embedded in paraffin wax. Transverse and tangential sections 8–10 μm thick were cut using a rotary microtome and then stained with 1% safranin O solution in 50% ethanol for examination with a light microscope.

Small samples of matchstick size were taken from each xylem sample, and macerated in Schultze's solution for a week at room temperature. The samples were then agitated gently to disintegrate individual fibers according to the method described by Berlyn and Miksche.[2] After maceration, lengths of 30 randomly selected fibers and vessel elements and the widths of 30 fibers and fiber lumens were measured. The lengths of vessel elements were measured from tip to tip including the tail.[3] These measurements and counts were made according to the methods prescribed by the IAWA Committee on Nomenclature.[4]

Tangential diameters of 30 vessels, and heights and widths of 30 rays were measured from the transverse and tangential microtomed sections. In addition, using these sections, the number of vessel elements per square millimeter and rays per millimeter were counted 30 times for the measurement of the cell densities. The different anatomical properties of the abnormal xylem in the vicinity of the enclosed bark and normal xylem were statistically analyzed by Student's *t*-test.[5]

Scanning electron microscopy observations

For scanning electron microscopy (SEM) observations, each xylem sample was fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and dehydrated in an ethanol series of 50%–95% for 20 min for each step, then three times in 99.5% ethanol, and then freeze-dried for 48 h. The samples were mounted on stubs and then coated with gold–palladium prior to examination with a Jeol JSM-5310 scanning microscope.

Results and discussion

Appearance of transverse disks of the canker

Figure 2 shows the transverse disk of the canker sample, which was cut from the stem near the ground. The exposed xylem is dark brown and has a decayed appearance. The bark wound caused by regular tapping was completely closed by callus formation, and then enclosed within the xylem. The enclosed bark could be seen clearly with the naked eye, appearing as darkened ring-shaped scratches on the transverse disks of the canker.

The discoloration and decay of the xylem tissues along the canker margin appeared to be extended tangentially from outside, following the tapping wound pattern. The affected xylem of the canker was extremely deformed. The wide growth zone of the xylem tissue bulged on both sides adjacent to the exposed xylem, where phloem tissues were also discolored and decayed.

The light tapping on the bark of the rubber trees caused a shallow wound in the phloem,[6] but the wounded bark enclosed within the xylem was thought to be caused by heavier tapping that partially removed the cambium.[7] The absence of cambium cells prevented the normal renewal of the bark as well as the renewal of xylem cells. In this case, the callus formed from the activity of meristematic tissue at the edges of the injured area covered the wound zone, resulting in the inclusion of a thin layer of bark by xylem formation.

Abnormal features of xylem in the vicinity of enclosed bark and around the wide growth zone of canker

The enclosed bark and xylem tissue were integrated, and in such areas an abundance of cells commonly appeared with thickened and lignified walls along the site. The xylem was aligned with plugged cells, and vessels surrounded by plugged cells were observed in the upper part of the integrated site (Fig. 3a, b). Laticifers and sieve tubes of the enclosed bark were not observed.

The scar tissue was seen underneath the wound periderm cells, which were regularly arranged in a radial pattern (Fig. 3c). The periderm cells had thickened cell walls and the lumens were plugged with latex (Fig. 3d). The latex plug was thought to be composed of tannin compounds, which are often deposited in enclosed bark tissues of rubberwood.[8] On the opposite side, xylem attached to the scar tissue possessed ray and axial parenchyma cells that were also plugged with tannin (Fig. 3c).

Cells with thick, lignified walls in the enclosed bark, present mainly along the tapping wound site, were substantively derived from phelloderm cells. According to Thomas et al.,[8] phelloderm cells have cellulosic walls in the initial stage, but in the later stage many of them became thick and lignified to form sclereids, whereas phellem cells had tannin-filled lumens. The disordered cells at the region where the bark was peeled off were heavily lignified, and the sclereids were also confined to the periphery. Such cell characteristics indicated that the development of hardy tissue presumably converted the scar or scab tissues in the enclosed bark. In the regeneration of rubberwood bark, the wound tissue was thought to consist of peridermal and some sclereid cells, which became scar or scab tissue.[9] Thomas et al.[8] also noted that the first periderm was functionless and pushed to the periphery, resulting in the development of shallow fissures, which later sloughed off.

Xylem elements in the vicinity of the enclosed bark exhibited abnormal growth where vessel elements were nearly spherical in shape, and were significantly fewer and smaller in length and tangential diameter, as well as more numerous than normal vessels (Table 1). The tangential diameter of rays was significantly larger than that of normal xylem, whereas no significant difference in xylem fibers was observed. In addition, diffuse-in-aggregates and reticulate-type axial parenchyma of rubberwood was not obvious in these areas.

Differentiation of fewer and narrower xylem vessels is also known as a common phenomenon in wounded xylem.[10] This characteristic is also seen in xylem formed on Nectria canker of *Fraxinus mandshurica*.[11] Meanwhile, enlarged ray

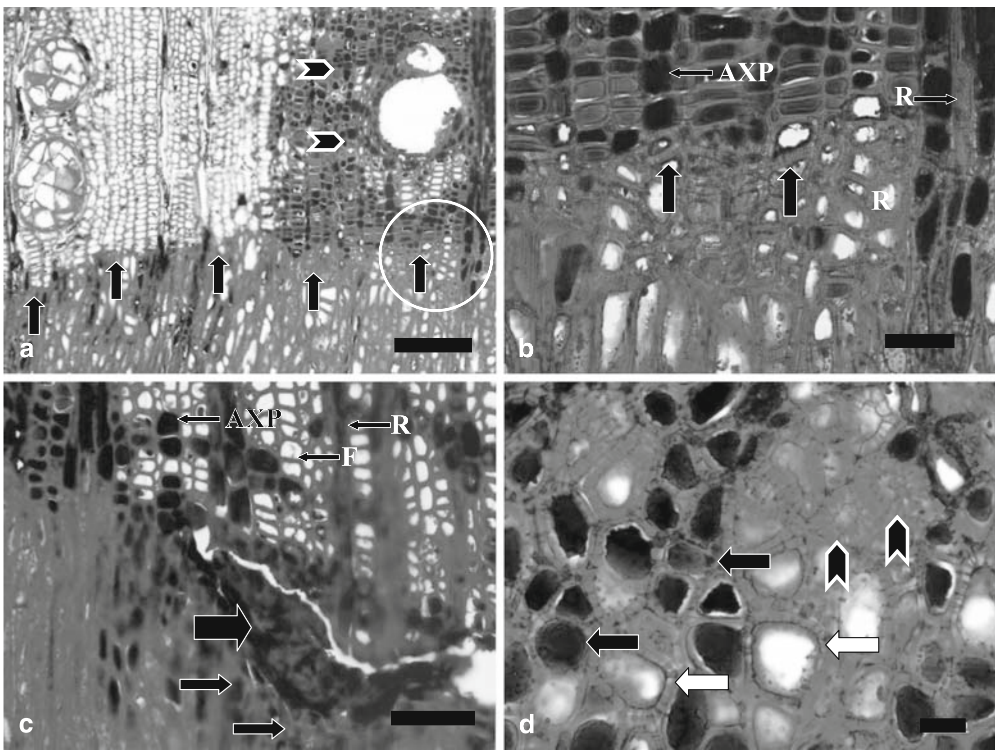

Fig. 3a–d. Microscopic features of the integrated region of enclosed bark and xylem and around the wide growth zone of canker. **a** Integrated tissue of enclosed bark and xylem. Note the abundance of cells with thickened and lignified walls present at the integrated site (*arrows*) and xylem with vessels surrounded by many plugged cells (*arrowheads*). **b** Close-up view of the integrated site taken from the *circled area* of Fig. 3a. Note ray (*R*) and axial parenchyma cells (*AXP*) and cells with thickened and lignified walls present at the integrated site (*arrows*). **c** Darkened scar tissue (*large arrow*) remaining at the integrated site. Note the scar tissue attached beneath the wound periderm (*small arrows*), whereas the opposite side is attached to xylem containing fibers (*F*), ray (*R*), and axial parenchyma cells (*AXP*). **d** Wound periderm cells filled with tannin (*black arrows*), and thickened cell walls (*white arrows*). Note the disordered sclereids (*arrowheads*) present around the wound periderm. *Bars*, **a** 200 μm; **b**, **c** 100 μm; **d** 20 μm

Table 1. Comparison of anatomical properties of xylem in the vicinity of the enclosed bark and normal xylem of rubberwood by Student's *t*-test

Xylem element	Vessel element diameter (μm)	Vessel element length (mm)	Number of vessels (mm^{-2})	Ray width (μm)	Ray height (μm)	Number of rays (mm^{-1})	Fiber length (μm)	Fiber width (μm)	Fiber lumen width (μm)	Fiber double wall thickness (μm)
Xylem in vicinity of enclosed bark[a]	121.17 ± 30.18	386.33 ± 79.15	2.97 ± 2.03	49.67 ± 7.30	843 ± 246.94	9.67 ± 1.06	1227 ± 111.5	26.06 ± 4.25	18.55 ± 3.54	22.31 ± 3.78
Normal xylem[a]	247.33 ± 44.08	669 ± 84.34	5.73 ± 2.85	43.58 ± 6.55	965 ± 251.6	9.13 ± 1.31	1209 ± 126.2	27.75 ± 3.35	18.30 ± 3.22	23.02 ± 3.15
ANOVA	167.338*	179.174*	18.794*	11.529*	3.593	3.013	0.330	2.912	0.084	0.636
Student's *t*-test	12.719*	13.386*	4.262*	3.395*	–	–	–	–	–	–

ANOVA, Analysis of variance
* $P < 0.01$
[a]Data given as mean ± standard deviation

cells of the xylem are involved in the healing process of bark wounds, as Thomas et al.[8] reported that enlarged ray cells exist near the cut end of the renewed bark of rubberwood. We assumed that vascular rays played an important role in the process of wound healing, and that the enlarged rays at the wound site were the main contributors to the formation of phellem and phelloderm division.

Abnormal forms of xylem in the vicinity of the enclosed bark were also visible around the wider growth zone adjacent to the canker. In this area, disoriented axial cells were more obvious in tangential views (Fig. 4a), while the cells shape was warped toward the canker zone (Fig. 4b). An irregular orientation of xylem elements was commonly observed in transverse views (Fig. 4c). These evidences show that the cambium in the immediate vicinity of the canker actively grew to generate cells for closing the canker. Such disoriented axial cells have frequently been investigated in xylem formed after wounding when canker disease is present, for instance, canker formation by inoculation in *Fraxinus mandshurica*.[11]

The presence of the wide growth zone adjacent to the canker as shown in Fig. 1b, indicates the active formation of cells to close the wound area of the canker tree. Xylem in the vicinity of enclosed bark taken from the wide growth zone area exhibited disoriented axial cells (Fig. 4a–c) resulting from the regeneration of callus tissue to close the wound. This phenomenon was in accordance with the statement of Blanchette,[12] that when a tree was wounded, the cells were actively generated to close the wound. It was noted that callus tissues were generally formed around the margins of the wound by the vascular cambium, and small wounds might be closed within one to several years, whereas large wounds took decades to be completely closed. Novitskaya[13] also reported that the axial cells of wound wood of beach trees had an irregular winding shape in tangential sections, indicating the different cell orientations from those in the undamaged trunk area. It was pointed out that the axial cells of the wound wood were considerably shorter than those of normal wood as a result of shortening of fusiform initials in the regenerated cambium.

The portion of xylem very close to the decayed area was observed to comprise a number of types of xylem cells, particularly rays and axial parenchyma cells, often containing gum/deposit as well as vessels plugged with tyloses (Fig. 4d). Vessel elements of the healthy tissues are commonly filled with tyloses in the heartwood of rubberwood. Most of the vessel elements at the canker margin showed the presence of tyloses even in sapwood, and the cell lumen surfaces of the vessels were free of gum/deposits. It appears that the tree form such a barrier in an effort to isolate the infected tissues, and to prevent the spread of the pathogen through the tissues. It has been suggested that the xylem elements containing tyloses obstruct the movement of fungal hyphae, and that the abundance of gum in axial parenchyma cells acts as a radial barrier to fungal development.[12] Despite the formation of these barriers, it appeared that the pathogen could infest and grow in the xylem tissues, causing their degradation.

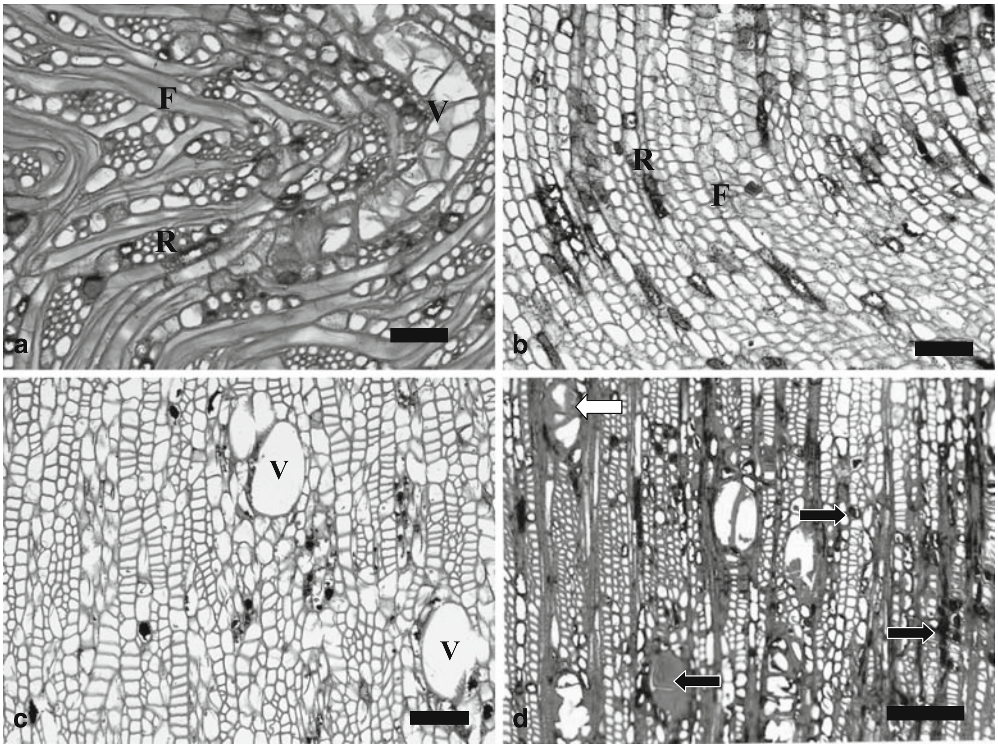

Fig. 4a–d. Abnormal forms of xylem around the wide growth zone and xylem adjacent to the canker margin. **a** Tangential view of disoriented axial cells consisting of vessel (*V*), fiber (*F*), and ray (*R*). **b** Xylem growth warping toward the canker zone. **c** Transverse view of the irregular orientation of xylem cells. **d** Xylem with vessels, rays, and axial parenchyma cells containing gum/deposit (*black arrows*) and vessels plugged with tyloses (*white arrow*). *Bars*, **a** 100 μm; **b**, **c**, **d** 200 μm

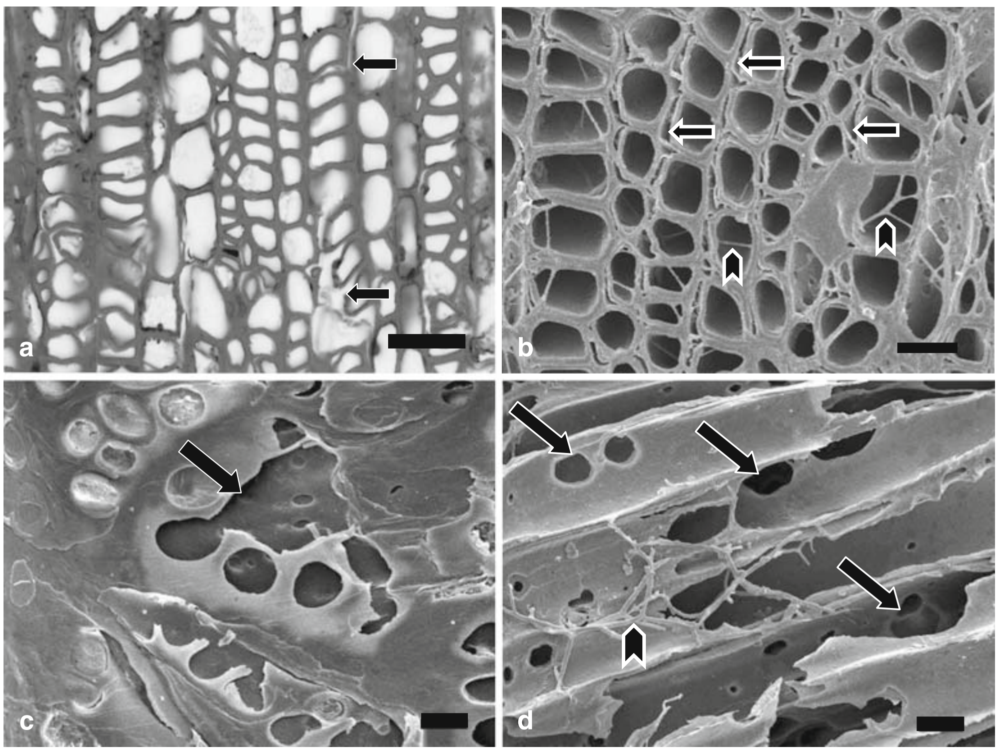

Fig. 5a–d. Decay features of xylem in the canker margin. **a** Separation among cells due to the partial degradation of the middle lamella (*arrows*). **b** General view of colonization of fungal hyphae causing xylem cell degradation (*arrowhead*) and separation among cells along middle lamella (*arrows*). **c** Rounded erosion of vessel pits, followed by coalescence of the rounded pits, caused the vessel wall to erode (*arrow*). **d** Colonization of hypha (*arrowhead*) and pit erosion in parenchyma cell walls (*arrows*). *Bars*, **a** 50 μm; **b** 20 μm; **c**, **d** 10 μm

Decay features of xylem in the canker margin

On the transverse sections of the canker margin, decayed xylem was clearly observed microscopically. Fungal hyphae colonizing the lumen of xylem cells could be observed under light microscopy and scanning electron microscopy (Fig. 5a, b). In transverse sections, separation among xylem cells due to partial degradation of the middle lamella was observed mainly on fibers (Fig. 5a).

The fungus was clearly observed causing disruption of the cell walls, and their concentric degradation, starting from the lumen surfaces (Fig. 5c, d). Hyphae penetrating mainly through the pits of xylem cells caused them to become distorted and enlarged. In view of the degradation of the wood, including separation among cells along the middle lamella and erosion of the cell pits, it was assumed that decayed xylem was caused mainly by white-rot fungal attack.[14,15]

Many pathological studies have also reported that Oomycetous fungi, *Phytophthora palmivora* and *Pythium vexans*, either alone[1] or together,[16] are causal agents of peach canker in rubber trees. According to Biggs,[17] pathogenic fungi belonging to the Oomycetes, Ascomycetes, and Deuteromycetes, which cause stem canker, are restricted to bark and xylem tissue that succumb due to the effects of toxins or secreted enzymes. Most of these fungi invade and colonize the xylem tissue in the vicinity of the canker without causing decay. However, the rubberwood canker observed in the present study exhibited a longitudinal canker with exposed xylem, which was discolored and decayed. We assumed that decay fungi might have infected the stem as a secondary invader through a large opening in the rubberwood canker, as reported in the canker trees of other species.[18]

We considered that the bark was repeatedly tapped while the trees were still actively producing latex, and that canker did not occur on the stems of rubberwood. Apparently, the latex acted as an antifungal compound[19] and played an important role in restricting fungal infection to the wounded tissues, so that the fully developed surface callus could completely close the bark wound when wound cambium had formed.[20,21] Canker disease occurring on the stem of rubberwood was assumed to be initiated by unhampered pathogen infection into the open wound of the tapping bark when the trees were not producing latex or when its latex yield was extremely low.

To confirm whether the fungus is a causal agent of xylem decay of rubberwood canker, a fungus identification and inoculation experiment, and evaluation of the process of infestation of living trees, should be undertaken in further studies.

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