Phyton (Horn, Austria)	**Vol. 51**	Fasc. 2	231–244	20. 12. 2011

Structural and Dimensional Changes in the Cambium of Tapping Panel Dryness Affected Bark of *Hevea brasiliensis*

By

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With 2 Figures

Received February 1, 2010

Accepted November 10, 2010

Key words: Alignment of cambium, ethephon, *Euphorbiaceae*, fusiform cambial cells, *Hevea brasiliensis*, tapping panel dryness, vascular rays.

Summary

PRAMOD S., THOMAS V. & RAO K. S. 2011. Structural and dimensional changes in the cambium of tapping panel dryness affected bark of *Hevea brasiliensis*. – Phyton (Horn, Austria) 51 (2): 231–244, with 2 figures.

The structural changes associated with the cambium of bark area affected by tapping panel dryness (TPD) have been studied in *Hevea brasiliensis* and compared with those of unaffected bark area of the same tree and that of TPD unaffected (healthy) trees. A number of structural deformations and dimensional changes occurred in the cambial zone cells including the altered cambial activity in the affected area. The cambium is nonstoried with elongated fusiform initials having actively growing tips enclosing uni or multiseriate rays. The anatomical features of cambium in relation to the structural changes in the affected trees were studied by observing the alignment of cells in the cambial zone. The changes in the cambial zone of affected area of bark include shortening of fusiform cambial cells, increase in the number of cambial rays, number of terminal cell of ray, width of ray and number of cell layers constituting the cambial zone. In the affected bark, fusiform cambial cells underwent transformative division leading to increment in the width and height of

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cambial rays. While in the TPD affected trees with warty outgrowth, the height of ray cambial cells decreased drastically and showed an increase in their width and density. The effect of ethylene in triggering the structural symptoms associated with TPD has also been studied as the ethephon application is a common practice in rubber plantations to stimulate the latex yield. In healthy trees, ethephon stimulation enhanced the cell divisions and length of fusiform cambial cells. The stimulation on the affected bark resulted in triggering of structural aberrations in the cambial zone cells. The effect of permanently altered cambial activity on the differentiation of laticifier system with respect to TPD has been discussed.

Zusammenfassung

PRAMOD S., THOMAS V. & RAO K. S. 2011. Structural and dimensional changes in the cambium of tapping panel dryness affected bark of *Hevea brasiliensis*. [Änderungen der Struktur und Größe des Kambiums durch von „tapping panel dryness (TPD)“ beeinflusster Rinde von *Hevea brasiliensis*. – Phyton (Horn, Austria) 51 (2): 231–244, 2 Abbildungen.

An *Hevea brasiliensis* wurden die strukturellen Veränderungen des Rinden-Kambiums, die durch “tapping panel dryness (TPD)” hervorgerufen werden, untersucht. Zum Vergleich diente entweder die unbeeinflusste Rinde desselben Baumes, oder es wurden andere, von TPD unbeeinflusste (gesunde) Bäume untersucht. Sowohl die Aktivität der Kambialzone änderte sich, als auch eine Reihe von strukturellen Deformationen und Größenänderungen tauchten in Zellen der Kambialzone des beeinflussten Bereiches auf. Das Kambium hat verlängerte spindelförmige Initialzellen, die aktiv wachsende Spitzen mit uni- oder multiseraten Strahlen beinhalten. Durch die Beobachtung der Anordnung der Zellen in der Kambialzone konnten die anatomischen Besonderheiten des Kambiums im Zusammenhang mit den strukturellen Veränderungen bei den beeinflussten Bäumen untersucht werden. Die fusiformen Kambialzellen der Kambialzone des beeinflussten Bereichs waren kürzer, gleichzeitig erhöhte sich die Anzahl der Kambialstrahlen, die Anzahl der Endzellen der Strahlen, sowie die Breite der Strahlen und die Anzahl der Zelllagen, die die Kambialzone bilden. In der beeinflussten Rinde kam es, durch Zellteilungen hervorgerufen, zu einer Zunahme der Breite und der Höhe der Kambialstrahlen. Während bei TPD beeinflussten Bäumen mit warzigen Auswüchsen die Höhe der Strahlkambialzellen drastisch abnahm und eine Zunahme an Dichte und Breite zeigte. Weiters wurde der Effekt von Ethen als Auslöser der strukturellen Symptome verbunden mit TPD untersucht: Etephon Applizierung ist eine normale Praxis in Gummi Plantagen, um die Latex Ausbeute zu steigern. In gesunden Bäumen bewirkt Ethephon eine Steigerung der Zellteilungsaktivität und eine Zunahme der Länge der spindelförmigen Kambiumzellen. Eine Stimulierung von TPD betroffenen Bäumen führte zu einem Anstieg der strukturellen Aberrationen in den Zellen der Kambialzone. Der Effekt der permanent veränderten Kambiumaktivität auf die Differenzierung von milcherzeugenden Systemen im Zusammenhang mit TPD wird diskutiert.

Introduction

Natural rubber, an indispensable industrial raw material, is obtained primarily from *Hevea brasiliensis*, a deciduous tropical tree indigenous to the Amazon river bank in Central and South America. The product of commercial importance obtained from *Hevea* is latex, which is derived from latex vessels by systematic wounding of bark on the tree trunk (THOMAS & al. 1995). The economic life span of the rubber tree is for more than 25 years during which both virgin and renewed bark are severed several times for latex. However, the over exploitation of trees by intensive tapping leads to a severe syndrome known as Tapping Panel Dryness (TPD) by which the tree become unproductive for latex yield (SIVAKUMARAN & al. 1988, JACOB & KRISHNAKUMAR 2006). Cessation of latex flow following tapping is the first visible symptom of TPD. The high yielding clones of *Hevea* are more susceptible to TPD. Based on the morphological symptoms on bark surface, the bark of TPD affected trees is classified as bark with warty outgrowth and without warty outgrowth. Even though different reasons were attributed to this century old serious problem till now no remedy has been achieved in TPD affected trees other than a few management practices through grafting technique (PREMAKUMARI & al. 1996b) or debarking of the unproductive bark (THOMAS & al. 1998). The bark and wood of affected trees show many morphological, structural and biochemical changes such as formation of occluded xylem, warty bark outgrowths, bark flaking, abundant sclereids in the bark, blocking of latex vessels with tylosoids, intense activities of the respiratory enzymes and other enzymes such as acid phosphatase, ATPase etc. (GOMEZ 1982, FAY & JACOB 1989).

It has been reported that the pattern of cambial activity in the affected trees varies considerably compared to that of healthy trees (THOMAS & al. 2002, 2006). The structural characteristics associated with bark and wood are the reflection of the activity of vascular cambium. Hence, the structural deformations associated with the TPD would be the result of reflection of cambial activity from its normal rhythm.

It has also been reported that the level of endogenous ethylene produced as a result of tapping or the exogenous application of ethylene on the bark can enhance the incidence of TPD together with associated histological changes in the differentiation of cambial derivatives including latex vessels (CHRESTIN 1984). However, a detailed investigation on the cambial activity and behavior of cambial zone cells in *Hevea brasiliensis* is lacking. Therefore, the present study has been initiated to unravel the associated structural and dimensional changes with cambium of both healthy and TPD affected bark of *Hevea* following application of ethephon.

Materials and Methods

Six mature trees of *Hevea brasilensis* (Clone RRII 105, planted in 1981) of both healthy (untapped) and TPD affected on rest were selected from of experimental area Rubber Research Institute of India at Kottayam in Central Kerala, India. For the study of behavior of cambial cells in healthy and TPD affected trees, cambium samples along with sap wood and inner bark were collected from the main trunk with the help of a chisel and hammer. In case of TPD affected trees, samples were collected from two areas on the bark, the affected area and unaffected area above the affected one. For the experiment, ethephon was dissolved in coconut oil which acts as an adsorbent medium. The outer periderm layer was removed from the bark of healthy and TPD affected trees and 5% ethephon was applied as a complete band of 2 inch width with an interval of 15 days for 3 months. Samples of cambium along with sap wood and inner bark were then collected from stimulated and non-stimulated bark area of healthy trees and from stimulated affected area, unaffected area and non-stimulated affected area of affected trees. Samples were fixed in formalin-acetic acid-alcohol and then processed for paraffin embedding using conventional methods (BERLYN & MIKSCHE 1976). Samples were dehydrated in Tertiary Butyl Alcohol series and finally embedded in paraffin with 58–60°C melting temperature. Transverse sections (TS) and tangential longitudinal sections (TLS) of 10 µm thick were cut on a Reichert Histo STAT rotary microtome. Sections were stained with Tannic acid-Ferric chloride-Lacmoid (CHEADLE & al. 1953) for cambium and Oil red O (OMMAN & REGHU 2003), Sudan IV (PREMAKUMARI & al. 1996a) and Nile blue sulphate (GAHAN 1984) for latex vessels.

Number of cambial cell layers and width of cambial zone were counted and measured from transverse sections. The dimensions of fusiform cambial cells and ray cambial cells were measured from tangential longitudinal sections. All the Observations, measurements and photographs were taken by using a research microscope (Leitz, Diaplan, Germany) attached with a Digital camera (Leica D320, Germany) and Leica Qwin V3 image analyzing system. 50 random measurements were taken for each anatomical parameter and Student *t*-test was used to determine statistically significant difference at 0.05 confidence level using Sigmastat software (Version 3.5, San Jose, CA, USA).

Results

Hevea brasiliensis is a tropical deciduous tree exhibiting seasonal cambial activity. The cambium is nonstoried with elongated fusiform initials and multi-seriate ray initials (Fig. 1A). The rays consist of a single terminal cell on either ends. A number of structural changes occur in the TPD affected bark which reflects adversely in its productive nature. The detailed investigation on the structural aspects of cambium revealed several ways of deformations in the cells in response to TPD. The basic alignment of cambial zone cells in the affected area was changed compared to that of unaffected bark area of the same tree.

The related aspects on the activity of cambium such as number of cell layers constituting cambial zone, dimensions of fusiform initials and cambial rays, number of cambial rays per unit area, number of terminal cells in the rays in the healthy tree, affected and unaffected areas of TPD trees and those trees after ethephon treatment are presented in the Tables 1, 2 and 3.

Cambium in Healthy Trees

In healthy untapped trees, the cambial zone consists of 8–10 cell layers with a mean radial width of 87 µm. The mean length and width of fusiform cambial cells were 761 and 29 µm respectively. The ray cambial cells are bi- to multiseriate with a mean height and width of 408 and 48 µm respectively. The mean density of ray cambial cells was 11 (Table 1).

Table 1. Characteristics of cambial cells in untapped and tapped healthy trees of *Hevea*.

Observations	Healthy untapped bark	Tapped unaffected bark	P Value
Number of cambial cell layers	9 ± 1	8 ± 1	0.653
Width of the cambial zone (µm)	87 ± 16	80 ± 10	0.489
Length of fusiform cambial cells (µm)	761 ± 86	665 ± 76	0.026*
Width of fusiform cambial cells (µm)	29 ± 4	26 ± 2	0.001*
Height of cambial rays (µm)	408 ± 58	336 ± 46	0.018*
Width of cambial rays (µm)	48 ± 6	38 ± 6	<0.001*
Number of cambial rays per 1mm^2 area	11 ± 1	12 ± 1.5	1.000

Note: * Significant changes following student t-test ($\alpha = 0.05$). p value: probability value.

In unaffected bark of tapped trees, the cambial zone was narrow with 6-8 cell layers (Fig. 1E). A reduction in dimensions of fusiform and ray cambial cells was also observed (Table 1). The mean density of rays in the cambial zone was more compared to that of untapped bark.

Cambium in TPD Affected Bark

In affected trees, bark with normal outward appearance, the width of cambial zone was reduced critically with 3–5 cell layers compared to that of untapped and unaffected bark (Fig. 1D, Table 2). The dimensions of fusiform cambial cells reduced, whereas, the cambial rays showed a reduction in height and increment in width as compared to that of unaffected bark. Transformative divisions of fusiform cambial cells leading to the development of cambial rays were prominent in the cambial zone (Fig. 1B). The density and number of terminal cells of cambial rays also increased significantly (Fig. 1C).

Warty outgrowth is one of the symptoms during the advanced stage of TPD. The changes in the cambium of bark with warty outgrowth were much severe as compared to that of TPD affected bark with normal outward appearance. In the warty bark area, fusiform cambial cells and cambial rays became shorter and wider (Table 2).

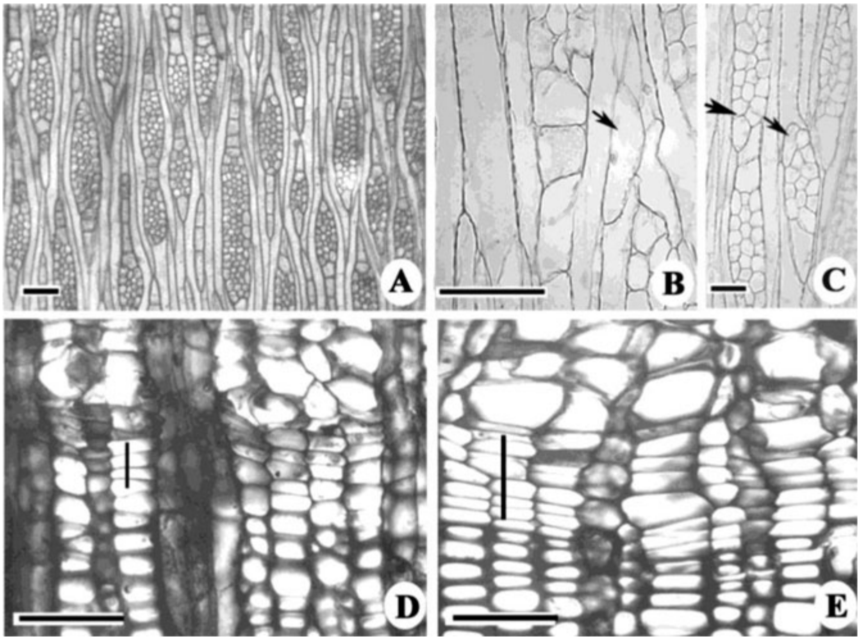

Fig. 1. Tangential longitudinal (A- C) and transverse sections (D&E) of cambium in healthy and TPD affected *Hevea* trees. A: The non-storied cambium of healthy tree showing axially elongated fusiform initials and short isodiametric ray initials. B: The cambium in the affected bark area showing transformation of fusiform initials into ray initials (arrow). C: Ray initials derived from transformation division of fusiform initials in the TPD affected bark (arrows). D: Narrow cambial zone (vertical line) in the affected bark area showing 3–5 layers of fusiform cambial cells. E:The wide cambial zone (vertical line) in the unaffected bark showing 6–8 layers of fusiform cambial cells. Scale bar = 50 μm.

Table 2. Cambial characteristics in TPD affected bark of *Hevea* with normal outward appearance and with warty outgrowth.

Observations	Bark with normal outward appearance	Bark with warty outgrowth	P Value
Number of cambial cell layers	5±0.8	5±1	0.625
Width of the cambial zone (μm)	53±4	53±5	0.525
Length of fusiform cambial cells (μm)	616±70	408±89	0.040*
Width of fusiform cambial cells (μm)	24±4.5	21±4	0.153
Height of cambial rays (μm)	360±118	282±63	0.029*
Width of ray cambial cells (μm)	45±4	55±	<0.001*
Number of cambial rays per 1mm^2 area	15±0.8	15±1	1.000

Note: * Significant changes following student t-test (a=0.05). p value: probability value.

Cambium in Ethephon Treated Bark

Ethephon treatment on the bark of both healthy and TPD affected trees resulted in changes in the pattern of cambial cell division and differentiation of their derivatives. In healthy trees, even though stimulation enhances cell divisions there was no noticeable change in the orientation of both fusiform and ray cambial cells. The tangential longitudinal sections showed that the terminal cells of rays undergo periclinal divisions leading to the increment in number of cells at vertical ends of rays. The ethephon treatment on healthy trees has enhanced the periclinal divisions in cambial zone cells and tissue differentiation of xylem and phloem. The length of fusiform cambial cells decreased after the treatment. There was no significant difference in the height of cambial rays, but a considerable increment in their width was observed following ethephon treatment (Table 3). The ray parenchyma in phloem appeared to undergo anticlinal divisions giving rise to bi-to multiseriate rays. The ray parenchyma in the xylem and phloem adjacent to treated cambium proliferated extensively and filled with polyphenol compounds. Also, there was a little increment in density of rays ranging from 9–13 rays for unit area. These results indicate that ethephon treatment can alter the cambial activity in healthy trees.

Table 3. Characteristics of cambial cells in control and ethephon treated area in the healthy untapped bark of *Hevea*.

Observations	Control bark	Stimulated bark	P Value
Number of cambial cell layers	9±1	7.6±1.3	<0.001*
Width of cambial zone (μm)	87±13	92±15	0.237
Length of fusiform cambial cells (μm)	724±85	683±92	0.369
Width of fusiform cambial cells (μm)	29±0.4	29±0.4	0.970
Height of ray cambial cells (μm)	410±82	480±72	0.217
Width of ray cambial cells (μm)	48±8	57±8	0.006*
Number of cambial rays per 1mm^2 area	11±1	12±2	0.388

Note: * Significant changes following student t-test (a = 0.05). p value: probability value.

In TPD affected bark with normal outward appearance, the ethephon treatment resulted in the reduction in number of cambial cell layers in the range of 3–5 (Table 4). On the phloem side, cambial zone cells showed differentiating elements mostly parenchyma rather than sieve elements and companion cells (Fig. 2A). Both fusiform and ray cambial cells became shorter in the treated area. The transformative divisions resulting in transformation of fusiform cambial cells into ray cambial cells were more in the treated cambium. There was a high density of ray cells for unit area in the range of 17–21 rays compared to that of control bark with 12–15 rays (Fig. 2B). The ethephon treatment also resulted in division of terminal ray cambial cells resulting more number of rays with multiple terminal cells (Fig. 2B).

Table 4. Characteristics of cambial cells in control and ethephon treated area in the TPD affected bark of *Hevea* with normal outward appearance.

Observations	Control bark	Stimulated bark	P Value
Number of cambial cell layers	5 ± 0.9	3.8 ± 0.6	< 0.001*
Width of cambial zone (µm)	55 ± 13	45 ± 12	0.009
Length of fusiform cambial cells (µm)	616 ± 70	430 ± 57	< 0.001*
Width of fusiform cambial cells (µm)	25 ± 4	24 ± 34	0.530
Height of ray cambial cells (µm)	352 ± 80	245 ± 51	0.025*
Width of ray cambial cells (µm)	44 ± 4	53 ± 6	0.010
Number of cambial rays per 1mm^2 area	15 ± 2	19 ± 1	0.011*

Note: * Significant changes following student t-test (a = 0.05). p value: probability value.

Table 5. Characteristics of cambial cells in control and ethephon treated area in the TPD affected bark of *Hevea* with warty outgrowth.

Observations	Control bark	Stimulated bark	P Value
Number of cambial cell layers	6.2 ± 0.9	7.7 ± 1	< 0.001*
Width of the cambial zone (µm)	59 ± 13	90 ± 16	< 0.001*
Length of fusiform cambial cells (µm)	696 ± 96	427 ± 98	< 0.001*
Width of fusiform cambial cells (µm)	25 ± 5	25 ± 2	1.000
Height of ray cambial cells (µm)	294 ± 92	323 ± 86	0.420
Width of ray cambial cells (µm)	66 ± 10	74 ± 9	0.003*
Number of cambial rays per 1mm^2 area	13 ± 0.8	16 ± 1	0.008*

Note: * Significant changes following student t-test (a = 0.05). p value: probability value.

In affected bark with warty outgrowth, the ethephon stimulation resulted in cambial cell divisions thereby the number of cambial cell layers increased in the cambial zone (Fig. 2D). The cambial cells divide in an irregular manner triggering changes in the orientation of cells in the cambial zone adjacent to phloem. The divisions lead to the transformation of fusiform cambial cells to ray cambial cells. The width of cambial zone increased measuring 60–124 µm. The fusiform cambial cells became shorter but width was comparable to that of control bark (Fig. 2C, Table 5). The height and width of the cambial rays increased compared to that of control bark. The density of rays in the cambial zone also increased following ethephon treatment. The rays also showed more number of terminal cells (Fig. 2C).

Discussion

The cambial cell division and differentiation are controlled by both external and internal factors (FAHN 1982, TANG & KOZLOWSKI 1984, KRISHNAKUMAR & al. 2001). On the other hand, the external factors can also influence the internal factors in controlling the rhythm of cambial activity (WAISEL & FAHN 1965). Increment in endogenous ethylene production is one

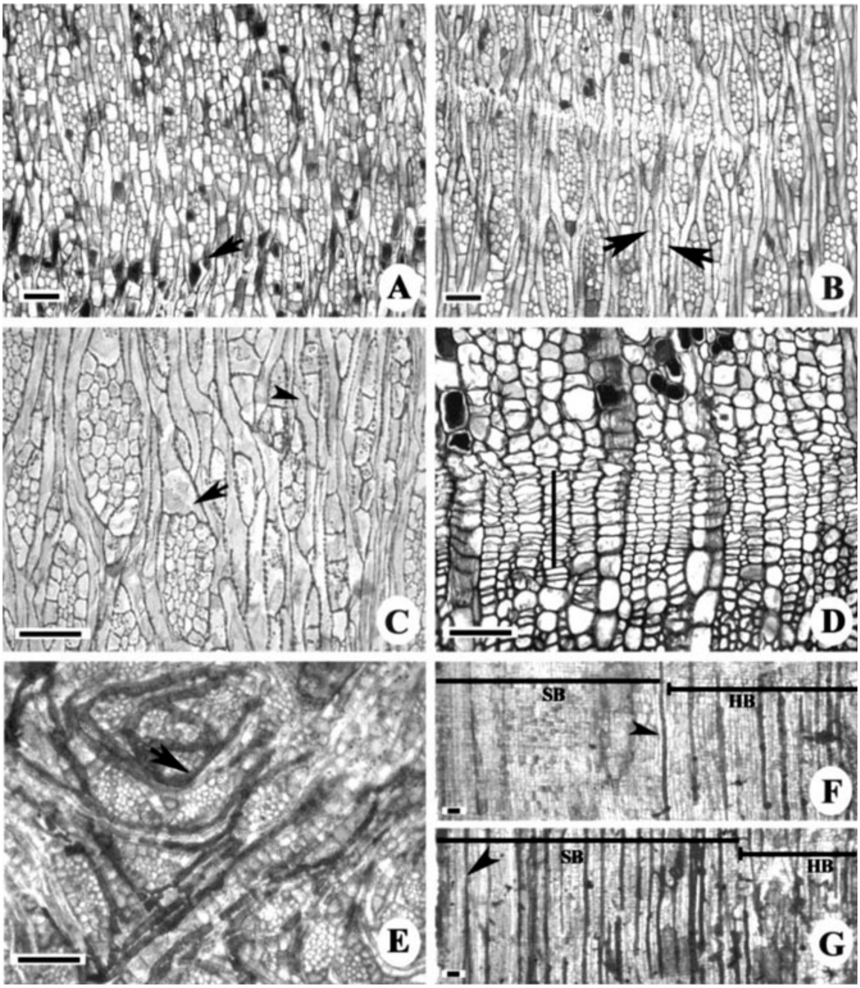

Fig. 2. Tangential longitudinal (A–C & E), transverse (D) and radial longitudinal sections (F, G) of cambium and bark of *Hevea*. A: Ethephon treated affected bark showing more number of parenchyma cells in the phloem (arrow). B: Stimulated affected cambium of normal TPD tree showing increase in frequency of rays and more number of terminal cells. Arrows indicate newly derived ray cells from fusiform initials through transformative division. C: Stimulated affected cambium in the bark of warty TPD tree showing short fusiform cambial cells and wide, short multiseriate rays with multiterminal cells (arrows). D: The transverse section of stimulated affected bark of warty TPD trees showing wide cambial zone (vertical line) with 6–9 layers of fusiform cambial cells. E: Tangential section of warty region of bark showing abnormal orientation of laticifiers and parenchyma cells (arrow). F: The affected bark showing reduction in latex vessel rows (arrow) in the soft bark (SB) adjacent to cambium and in hard bark (HB). G: The unaffected bark showing abundance of latex vessel rows (arrow) in the soft bark (SB) and hard bark (HB) zones. Scale bar = 50 µm.

of the major biochemical symptoms of TPD in *Hevea*. The normal course of tapping results in the increase of endogenous ethylene production which is directly proportional to the intensity of tapping. The increase in levels of ethylene either by tapping or exogenous application of ethylene in the bark may alter the cambial activity (YAMAMOTO & KOZLOWSKI 1987).

The changes in the normal course of cambial activity may reflect in the rate of cambial cell division, differentiation and basic alignment of fusiform and ray cambial cells. The rhythmic activity of cambium in *Hevea* is altered as a result of TPD (THOMAS & al. 2006). These authors also suggested that the changes occurring in the TPD affected bark area is found to be permanent. The density of cambial rays has increased considerably following the transformation of fusiform cambial cells into ray cambial cells in the affected area. During the normal development of cambium few transformative divisions of fusiform cambial cells into ray cambial cells occur by shortening and undergo repeated oblique, transverse or anticlinal divisions by which one of the daughter cells fails to undergo active intrusive growth and compete for space in the cambium (ROMBERGER & al. 1992). The shortening of fusiform cambial cell length and their transformation into ray initial may be part of a regulatory system whereby rays are initiated. This is effected through cell shortening, cell enlargement, division of the cells in different planes, intrusion of fusiform cambial cells into rays, partial or complete transformation of fusiform cambial cell into a ray. The width of cambial rays would be increased by the incorporation of cells at the upper and lower margins through a division in the fusiform initial adjacent to them (ROMBERGER & al. 1992). The vascular rays play an important role in the radial translocation of water and metabolites between the wood and the phloem.

The change in structure and activity of cambial cells might have a definite effect on the differentiation of phloem tissue which is responsible for radial and downward translocation of photosynthates, storage of metabolites and biosynthesis of latex. The orientation of latex vessels is more or less identical to the alignment of the sieve elements. The rhythm in the activity of cambium and the differentiation of laticifiers from the derivatives in definite intervals are inevitable in a deciduous tropical tree like *Hevea* and what signals would determine this rhythm is not yet understood. In the TPD affected and stimulated area of the bark the basic alignment of cambium has changed and a similar reflection has also been observed in the laticifiers differentiation. Since the activity of cambium in TPD affected trees has altered permanently under stress, the tissue including latex vessels differentiated from the fusiform cambial cells of the affected cambium exhibit the structural and functional abnormalities as observed by WU & HAO 1994. FAY & JACOB 1989 reported that the differentiation of latex vessels ceases in the TPD affected area of bark. The present study shows that the number and normal rhythm of latex vessel differentiation have been disrupted leading to an abnormal orientation of latex vessels which corresponds to the orientation of fusiform cambial cells in the cambium (Fig. 2E). Even though the latex vessels gave positive results with the stains used, they appear as unproductive due to the metabolic deficiency occurring in the inner part of the bark following to TPD (FAY & JACOB 1989).

The affected area is also characterized by narrow sieve elements and a reduction in its density compared to that of unaffected area. The reduction in the proportion of fusiform to ray initials in the affected cambium may be a defensive action of the tree under intense stress. This is because, the nutrient loss in *Hevea* is generally occurring through collection of latex present in the laticifiers cells and these cells receive their nourishment required for latex synthesis from sieve elements and surrounding parenchyma cells. It is also evident that there is a drastic reduction in the number of latex vessel rows derived from the affected cambium (Fig. 2F) than that of unaffected cambium (Fig. 2G). All these structural changes via altered cambial activity may be an adaptive mechanism of the plant to lower or prevent the loss of nutrients through the secondary metabolites like latex as these nutrients are mainly for the survival of the plant rather than secondary metabolite production. ESCHBACH & al. 1989 reported that a source sink imbalance, depletion of reserve assimilates, and /or interruption of their translocation through sieve elements in the tapping panel of affected area cause considerable slowing or stoppage of latex production as the *Hevea* carries out its vital function in priority. The corresponding increment in ray density through transformative divisions of the fusiform cambial cells may also be a part of defensive action as vascular rays play a key role in the process of wound healing and the response to stress particularly to high concentration of internal ethylene production compared to that of the axial parenchyma which are derived from fusiform cambial cells (ALONI 1995, THOMAS & al. 1995, SAUTER 2000).

The exogenous application of ethephon on the affected bark further demonstrates that the possible role of ethylene in alteration in the proportion of fusiform initial to ray initial ratio in the cambial zone and its effect on the normal distribution of axial and radial elements in the bark and wood. This may be the reason for the differentiating zone of affected area after stimulation shows a high reduction in the elements like sieve elements, laticifiers and companion cells and an increment in the density of parenchyma cells. This zone is observed to be similar to the barrier zone which forms in response to infection as well as mechanical wounding (TIPPET & SHIGO 1981) in many tree species and is characterized by a great amount of parenchyma cells and few conducting elements (TORELLI & al. 1994).

ALONI 1987 reported that the fusiform cambial cells when beginning to suffer from shortage in food supply are evidently able to divide unequally.

In this way the fusiform cambial cells can give rise to new ray cambial cells and thus to new radial routes for food supply since phloem rays are in direct contact with sieve elements. The cell division activity has been induced during ethephon stimulation and thereby there is a substantial increment in photosynthate utilization in the cambial zone. The usage of photoassimilates at the cambial site, which depends on the cell division activity that in turn is under control of hormonal and environmental stimuli (SAVIDGE 1996). The increased utility of photoassimilates may lead to shortage of food supply to fusiform cambial cells. Hence, the fusiform cambial cells can give rise to new ray cambial cells through transformative divisions so as to enhance the radial transport. Ethylene application can also increase the amount of ray tissue as observed in *Pinus* (YAMAMOTO & KOZLOWSKI 1987). The differentiation of ray parenchyma is important as they are responsible for the synthesis of various phenolic compounds (SHORTLE 1979) and are the source of tyloses and polysaccharide deposits in vessels (CHATTAWAY 1949).

The tannin accumulation in the cambium and differentiated cells in the affected and stimulated trees was found to be more. The high accumulation of tanniferous content may be due to a high or altered metabolism following stress (SWAIN 1965). The tannin accumulation also found near to cells destined to die shortly afterwards. The sieve tubes differentiated from the affected cambium are narrow (FAY & JACOB 1989) and the downward translocation is regulated by P-protein plugging and later inactivation by definitive callose deposition (PRAMOD 2007) which ultimately leads to the death of these elements. Mineral deficiency caused due to the lowered downward transport of carbohydrates and hormone growth regulators affect adversely the growth of cambium (KOZLOWSKI & PALLARDY 1997).

The stress caused by the ethephon application varies in individual trees and the degree of additional stress on TPD affected area may trigger both structural and functional aberrations. The transformation of normal TPD to warty TPD due to stimulation is another point of interest. Even though the structural deformation on cambium is specific to the affected bark areas, the affected tree shows certain physiological changes including poor pollen germination (SANKARIAMMAL & SARASWATHYAMMA 2004).

Once the cambial activity has been altered in the TPD affected area, cambium fails to regains its original state of activity. This is the reason why once a tree get affected with TPD, there is every chance for the reappearance of the symptom in trees recouped after a period of rest. As the structural changes occurring at the level of vascular cambium are extremely severe and remain permanent in the affected area, further studies are necessary to understand the cambial behavior at physiological and molecular level.

Acknowledgements

Authors are thankful to Dr. N.M. MATHEW, former Director of RRII for giving the permission to carryout the work at RRII. Thanks are also due to Dr. J. JACOB, Director of RRII and Dr. Y. ANNAMMA VARGHESE, Joint Director (Crop improvement) for encouragement.

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Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 2011

Band/Volume: 51_2

Autor(en)/Author(s): Pramod Sivian, Thomas Vinoth, Rao Karumanchi S.

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