

# **TROPICAL AGRICULTURAL SCIENCE**

Journal homepage: http://www.pertanika.upm.edu.my/

# The Dynamics of Radial Growth of Three Selected Tropical Tree Species Studied through Knife-cutting Method

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# ABSTRACT

Tropical trees which lack distinct growth rings have caused difficulty in estimating the growth rate of those trees. This has resulted in limited knowledge concerning tropical tree growth pattern and rate of increment. This study aimed to assess the radial growth and cell production rate of three selected tropical species, namely, Macaranga gigantea, Endospermum diadenum and Dipterocarpus costulatus, with different diameters at breast height. For this purpose, knife-cutting method was adopted in this study. A knife was inserted through the bark into the outer xylem of a tree to wound the cambium and remove immediately. Wood discs containing wound area were collected from living trees after a period of time. Transverse sections of 20-25 µm in thickness were obtained through sliding microtome and dehydrated in a graded series of ethyl alcohol before staining with safranin and fast green. Dibutyl phthalate xylene (DPX) was used as a mounting medium for preparation of permanent microscope slides. The species-related anatomical response to wounding was identified and used to define the time of marking. Results show that radial growth rate and cell production rate varied across species and tree sizes. M. gigantea and E. diadenum showed faster growth rates than D. costulatus, especially in small diameter classes. Meanwhile, D. costulatus had the lowest growth rate and cell production rate. Thus, both the pioneer species are thus considered to grow faster in smaller stem size than larger stem size, while the study succeeding species grow faster in larger stem size than smaller stem size.

Article history: Received: 29 August 2012 Accepted: 20 September 2012

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# **INTRODUCTION**

Tree growth consists of axial growth and radial growth (Nobuchi & Sahri, 2008).

ARTICLE INFO

It is difficult to estimate the growth rate of tropical tree species as most of these trees lack distinct growth ring (Ohashi et al., 2009). The absence of growth ring in tropical trees is believed as a result of less clear seasonality in the tropical areas (Rozendaal & Zuidema, 2011). The concept of continuous tree growth in the absence of distinct growth ring in tropical trees has been widely assumed (Lang & Knight, 1983; Lieberman et al., 1985, Whitmore, 1990; Worbes, 2002; Nobuchi & Sahri, 2008), and thus, leading to limited knowledge concerning tree growth pattern and rate of increment in tropical trees (Ashton, 1981). Furthermore, studies on the cell production rate in tropical trees are still very much limited. Among other, Nobuchi and Hori (2008) investigated and compared the radial growth and cell production rate between temperate and tropical trees but the tropical species was only represented species i.e., Eucalyptus camaldulensis.

Several methods have been used to detect the radial growth rate of tropical trees (Worbes, 1995, 2002). Ohashi *et al.* (2009) stated that the approaches to assess radial growth of tree can be categorized into direct measurement of radial growth increment, such as dendrometer measurement and cambial marking (Ohashi *et al.*, 2001; Baker *et al.*, 2002; da Silva *et al.*, 2002; Mariaux, 1967; Shiokura, 1989; Nobuchi *et al.*, 1995; Sass *et al.*, 1995), and detection of periodicity of wood formation, which includes isotope analyses (Evans & Schrag, 2004; Poussart *et al.*, 2004; Verheyden *et al.*, 2004). The knife-cutting method (Fujiwara, 1992), which was introduced to improve the pinning method (Wolter, 1968), is an excellent method to study cell production rate. It is a simple and easy method to implement in the field. It enables the timing of marking to be mark accurately and it can be used to study a range of small to large diameter trees (Nobuchi *et al.*, 1993). This method has been applied widely to temperate trees (Shimaji & Nagatsuka, 1971; Kuroda & Shimaji, 1983, 1984); however, it is still rarely applied to tropical trees (Veenin *et al.*, 2006).

The knife-cutting method causes small mechanical wound, which is used to define wood increment from the time of pinning without affecting the physiological process of a tree (Gričar, 2007). Hence, detail understanding of the anatomical response of a species to wounding is essential when implementing this technique (Nobuchi *et al.*, 1995; Kuroda & Kuyono, 1997; Mäkinen *et al.*, 2003; Schmitt *et al.*, 2000, 2004).

The objective of the study was to assess the radial growth and cell production rate of *Macaranga gigantea*, *Endospermum diadenum* and *Dipterocarpus costulatus* through knife-cutting method. The species selected consists of tropical pioneer and late-successional species with diameter less than 20 cm and more than 25 cm at breast height (DBH). The comparative approach was used to study the cell production and growth rate of the study species. This is relevant to understand the natural process of forest dynamics, in which the processes are parts of the natural order after disturbance.

#### MATERIALS AND METHODS

#### Study Sites and Trees

The experimental site was located at Ayer Hitam Forest Reserve in Selangor, Malaysia. The forest which belongs to tropical rainforest is one of the Dipterocarp inland forests found in the state of Selangor (Faridah-Hanum & Khamis, 1999). The species selected for this study were M. gigantea, E. diadenum and D. costulatus, which were grown in compartment 14. The investigated tree species ranged from very high-light demanding species to shade tolerant species. M. gigantea and E. diadenum are tropical early successional species, while D. costulatus belongs to tropical late successional species (Soerianegara & Lemmens, 1994; Sosef et al., 1998). All the sampled trees are evergreen throughout the year and the exact ages of the trees are unknown. Cambial marking was done to two trees for each species with different diameter ranges. Simple random sampling was adopted for the sampling technique. The descriptions of the sampled trees are shown in Table 1.

#### Meteorological Data

Throughout the research period, data on the monthly total rainfall and monthly mean temperature of the study site were collected from Pusat Pertanian Serdang Meteorological stations, Meteorological Department of Malaysia. The forest is categorized as rainforest area as it receives monthly rainfall of about 100 mm or more for the major part of a year. The highest rainfall was recorded in September 2010, with the total rainfall of 513.9 mm, whereas the lowest rainfall recorded during the observation period was in May 2011, with the total rainfall of 83.6 mm. The mean temperature ranges from 23.4°C to 27.7°C. The highest temperature was observed in May and June, while the lowest temperature was recorded in March. The maximum and minimum relative humidity was 79.1% and 66.9%, respectively.

### Knife-cutting Marking

A knife of 0.2 mm in thickness and 10 mm in width was used for marking. Knife was inserted through the bark into the outer xylem of a tree to wound the cambium

Species (Family)	Tree Number	Diameter at breast height DBH (cm)
Macaranga gigantea	MGJ 1	13.0
(Euphorbiaceae)	MGM 1	28.6
<i>Endospermum diadenum</i>	EDJ 3	16.7
(Euphorbiaceae)	EDM 3	28.1
Dipterocarpus costulatus	DCJ 2	12.2
(Dipterocarpaceae)	DCM 3	27.2

TABLE 1Descriptions of the sampled trees

(Nobuchi & Sahri, 2008). The knife was then removed immediately and the wound site was marked. This method was used on every sample tree on 26 August 2010.

# *Knife-cutting Sample Collection and Preparation*

Wood disc containing wound area was collected from every sampled tree on 18 July 2011. Wood blocks with marked parts were then obtained from wood discs by handsaw. The wood blocks were then incised by handsaw through the centre of long slit-like wound tissue, separating the wood blocks into two parts (Nobuchi & Sahri, 2008; Veenin et al., 2006). One part of the wood blocks was suitably trimmed to smaller size. Transverse sections of 20-25 µm in thickness were prepared using a Leica SM2000 microtome. The thin sections obtained through the sliding microtome were stained for light microscopy observation. A graded series of ethyl alcohol (30%, 50%, and 70%) was used for dehydration (Gričar, 2007). Double staining was adopted, in which sections were stained with Safranin overnight and followed by fast green (Nobuchi et al., 1995). Next, the sections were rinsed off with 95% ethyl alcohol several times to remove excess fast green. Clove oil was then dropped on every section produced. Meanwhile dibutyl phthalate xylene (DPX) was used as a mounting medium for the preparation of permanent microscope slides. A microscopy observation was carried out with Leica Leitz DMRB Microscope.

#### Softening of Wood Blocks

Since only *D.costulatus* has very hard xylem, the blocks were then softened in Ethylenediamine (Imai *et al.*, 1995; Kukachka, 1978). In this research, wood blocks were treated with 4% Ethylenediamine for about one week before transferring to 50% Ethylalcohol. The wood blocks were then washed with distilled water and ready for sectioning.

# Laboratory Analysis

The knife-cutting method was applied to calculate the rate of cell production in the study period (Nobuchi & Sahri, 2008). The total numbers of cells produced in the period between knife insertion and wood block collection were counted to investigate the cell production rate between the sample trees with different species and DBH. The method to calculate the cell production rate of the sample trees was the same as that used in the study by Nobuchi and Hori (2008), in which they compared the cambial activities between tropical and temperate trees. The outermost cell of S1 layer formation cells was used for measurements (Nobuchi & Hori, 2008). S1 formation cell during marking was the cell next to the innermost flattened cells to the pith side in the wound tissue. The initiation of S1 layer formation at the time of wood block collection was identified through polarized light microscope. The total number of cells between S1 layer formation cell line at the time of marking to the S1 layer formation cell line during wood block collected was counted as in Fig.1 (Nobuchi & Hori, 2008).

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Fig.1: Light micrograph of pinned area showing estimated cambial initials at the time of pinning experiment (26 August 2010) and cambial initials at the time of wood block collection (18 July 2011). Scale bar = 500  $\mu$ m.

In this study, all the sample trees have the same growing period. The pinning and wood block collection were done on 26 August 2010 and 18 July 2011, respectively, with a total of 326 days.

#### **RESULTS AND DISCUSSION**

#### Anatomical Characteristics of Growth Ring as a Reference Line

The characteristics of growth ring boundary, as a reference line, were investigated in transverse sections. Growth ring is necessary for the measurement of radial growth rate of trees in a given time (Nobuchi & Sahri, 2008).

The transverse sections of *M. gigantea*, *E. diadenum* and *D. costulatus* are shown in Fig.2. *M. gigantea* is diffuse-porous, while *E. diadenum* is semi-ring-porous. A detailed observation revealed that both species had growth rings. These two species showed distinct growth ring boundary in alteration of fibre cell walls thickness, which is useful as reference lines. The cells were radially flattened and had thicker fibre cell walls in the inner side of the boundary than on the outer side. However, D. costulatus did not have any distinct growth ring boundary as the cells structure changes were not visible. Sass et al. (1995) stated in their study that both Dryobalanops sumatrensis and Shorea *leprosula* from the *Dipterocarpaceae* family also do not form annual growth rings, and thus, determination of wood formation was difficult to make. Meanwhile, resin canals are abundant in this species and these are mostly formed in tangential bands, together with parenchyma, giving the impression of Kang Han, W., Hamami, M. S. and Nazre, M.



Fig.2: Transverse sections of: (a) *M. gigantean*, (b) *E. diadenum* showing the pattern of a growth-ring boundary with scale bar =  $200 \ \mu\text{m}$ . GR: growth ring (c) *D.costulata* showing absent of growth ring with scale bar =  $500 \ \mu\text{m}$ .

growth ring (Gottwald, 1958). Nonetheless, they often fail to run continuously around the stem and often become disappear (Sass *et al.*, 1995).

# Anatomical Characteristics of Wound Tissue

The transverse sections of the wound tissues induced by cambial marking in *M. gigantea*, *E. diadenum* and *D. costulatus* are shown in Fig.3. Nobuchi *et al.* (1995) and Ogata *et al.* (1996) stated that wound tissues can be divided into two zones and both these zones were focused in this study. In zone 1, cambial initials and cells in the differentiating zone had been directly affected by the knife-cutting method, as shown in Fig.4. The cambial and enlarging zone cells in this zone were crushed and the

cell wall formations were interrupted at the time of marking. They ceased their maturing and retained the cell wall organization at the time of marking, although the cells in this zone were deformed. The cells indicated by the arrowheads are the ones that were initiating  $S_1$  layer formation at the time of pinning (Nobuchi & Sahri, 2008). In zone 2, the cambial and enlarging zone cells were not crushed but indirectly affected by the marking method with plasma membrane intact and showed abnormal differentiation. In this study, zone 1 is termed "directly affected zone" and zone 2 is "indirectly affected zone".

Fig.5 shows the structural characteristics of directly affected zone, which was observed under a polarization microscope and conventional illumination. The crushed The Dynamics of Radial Growth of Three Selected Tropical Tree Species Studied through Knife-cutting Method



Fig.3:Transverse sections of wound tissue: (a) *M. gigantea,* (b) *E. diadenum,* and (c) *D. costulatus* Scale bar =  $500 \mu$ m.



Fig.4: Transverse sections of *E. diadenum* showing wound tissues with two zones marked. Zone 1: tissues directly affected by pinning injury; arrowheads indicate the deduced site of the initiation of  $S_1$  layer formation. Zone 2: tissues indirectly affected by pinning with tissue not crushed but showed abnormal differentiation; arrowheads indicate the deduced site of cambial initials. Arrow indicates the direction of the bark side. Scale bar = 500  $\mu$ m.



Fig.5: Transverse sections of directly affected zone, corresponding to zone 1 in Fig.3, observed under: (a) a polarization microscope, and (b) a conventional microscope in *E. diadenum*. Arrowheads indicate the deduced site of the initiation of  $S_1$  layer formation. EZ, SW indicates enlarging zone and secondary wall at the time of pinning, respectively.

cells were considered as the cells of cambial while enlarging zone cells as those cells that showed no birefringence (Ogata *et al.*, 1996). The boundary between the crushed and non-crushed cells, as shown by arrowhead in the Fig.5, was therefore judged to be the boundary between the enlarging zone (EZ) and the secondary wall thickening zone (SW) (Ogata *et al.* 1996). The position of S<sub>3</sub> layer formation was not clarified in this experiment.

The anatomical characteristics of indirectly affected zone showed a region of normal wood cells (NW), radially flatten cells (FC), and small diameter vessel elements (SV), from the pith side to the bark side, as shown in Fig.6. Meanwhile, callus-liked cells (c) were formed near the side of marking. The callus-like cells, which corresponded to the enlarging zone cells in the directly affected zone, were believed to have formed by ray parenchyma cells in order to fill the gap formed after knife insertion (Ogata et al., 1996; Nobuchi et al., 1995). The layers of the radially flat cells were considered to be the cambial zone at the time of marking. They remained undifferentiated after marking, and therefore, retained the structural characteristics of cambial zone. However, it is not clear whether the radially flat cells included all the cambial zone cells (Ogata et al., 1996). The cambial initials located above the layers of the radially flat cells were affected by the pinning but plasma membranes remain undestroyed. These cells are considered to have continued their physiological activity and to form wound tissues (Nobuchi et al., 1995). In the region where small diameter vessels were formed, there were points where the number of cell rows increased tangentially, as shown in Fig.6. The small vessels abnormally differentiated from cambial cells were considered to have been affected by cambial

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Fig.6: Transverse sections of *E. diadenum*: (a) near direct affected zone with callus tissue; (b) without callus tissues in zone 2 corresponding to indirectly influenced zone. NW: normal wood formed before marking; FC: radially flat cells showing undifferentiated cells affected by marking; C: callus-like cells corresponding to enlarging zone at the time of marking. Arrows show the location of anticlinal division. Scale bar =  $30 \mu m$ .

marking. Hence, this region is believed to have formed after marking (Ogata *et al.*, 1996). The increases of the cell rows were caused by the anticlinal division of cambial initials. Similarly, Nobuchi *et al.* (1995) stated that the line, which connected to the innermost point of anticlinal division, was theoretically considered as the location of cambial initials at the time of pinning. Thus, it adopted as the marker of the cambial initial at the time of pinning and used for the measurements.

The anatomical characteristics of M. gigantea and D. costulatus, which include their marking of cambial initials and the cell region having S<sub>1</sub> layer formation, are basically similarly to *E.diadenum*.

# Traumatic Resin Canals

In this study, traumatic resin canals were observed in *D. costulatus* but not in *M. gigantea* and *E. diadenum*. Fig.7 shows a

sample of the traumatic resin canals in *D. costulatus*. The traumatic resin canals were formed towards the bark side of the line of the estimated cambial initials at the time of marking. Kuroda and Shimaji (1983) and Shiokura (1989) used resin canals to estimate the position of cambial initials at the time of marking. In this study, the formations of traumatic resin canals were not used as the markers of cambial position because they did not reveal the exact position of cambium at the time of pinning.

# Cell Production Rate

Table 2 shows the total number of the cells produced in all the sampled trees during the observation period. The results showed that radial growth rate and cell production rate varied with species and tree sizes. Nabeshima *et al.* (2010) stated that the diameter growth of living trees may be different, depending on the tree species, size

Species	DBH (cm)	Radial growth (mm)	Rate of radial growth (µm/day)	Number of cells	Rate of cell production (Number/day)
Macaranga gigantea	$\leq 20$	4987.22	15.30	319	1.0
	> 25	3023.41	9.27	188	0.6
Endospermum diadenum	$\leq 20$	7643.13	23.45	386	1.2
	> 25	2207.72	6.77	121	0.4
Dipterocarpuscostulatus	$\leq 20$	1609.52	4.94	68	0.2
	> 25	2164.46	6.64	113	0.3

TABLE 2				
Radial growth	and 1	rate	of cell	production



Fig.7: Transverse sections of *D. costulatus* showing traumatic resin canals. Scale bar =  $200 \ \mu m$ 

and other tree-relating factors. Theoretical and ecophysiological studies also suggest that tree growth is closely associated with their sizes (Hubbard *et al.*, 1999; Enquist, 2002, Midgley, 2003; McDowell *et al.*, 2005; Nabeshima & Hiura, 2007) rather than with the age of trees (Mencuccini *et al.*, 2005; Matsuzaki *et al.*, 2005; Bond *et al.*, 2007).

During the research period, the two pioneer species, namely, *M. gigantea* and *E. diadenum*, showed higher radial growth rate and cell production rate compared to the non-pioneer *D.costulatus*, especially in small diameter classes. In particular, *E. diadenum* with DBH  $\leq$  20 cm showed the highest growth rate and cell production rate (number/days) in all the sample trees. On the contrary, *D. costulatus* had the lowest growth rate and cell production rate among all the species investigated. This might be due to the fact that most shade-tolerant species and late succession species have low growth rates (Franklin, 2003). Meanwhile, short-lived pioneers are required to grow faster as they normally cannot withstand and die in shade. Such species normally grow better in areas with the greatest crown exposure (Manokaran & Kochummen, 1992).

According to a research by Kohyama et al. (2003) on tree size and growth rate, as a tree diameter increases, the diameter growth rate will decrease. This phenomenon can be seen in M. gigantea and E. diadenum but not in D. costulatus. Both light-demanding pioneer species, with DBH less than 20cm, produced a high growth rate as compared to DBH that is more than 25cm, in which the growth rate drop rapidly. The growth advantage of the pioneers lost with the increasing in the tree size. However, Nabeshima et al. (2010) stated that the growth rate of tree would depend on DBH. The research found that the growth of Acer mono increased with DBH, while other species (such as Ostrya japonica, Quercus crispula and Magnolia obovata) increased their initial growth that subsequently declined with increasing DBH.

*D. costulatus* showed much slower growth rates than the other two species. In their study, Manokaran and Kochummen (1992) stated that shade-intolerant understorey species grew much slower than the other tree species. Short-lived shade intolerant species grow at a higher rate than long-lived shade tolerant species and this is believed to be due to their higher intrinsic growth rates at a given irradiance, as well as in high-light site characteristics in canopy gab (Swaine, 1994; Baker *et al.*, 2003). Trees that grow in the understorey of tropical forests usually lack lights, and resulting in very low diameter growth rates (Chazdon & Fetcher, 1984; Clark & Clark, 1999).

*D. costulatus* with DBH above 25 cm has higher radial growth rate than tree with DBH smaller than 20 cm. This is due to the fact that when trees grow to a certain canopy level, they grow more rapidly to compete for survival. Manokaran and Kochummen (1992) documented that individual canopy species, especially Dipterocarps, are more likely to grow at relatively fast rates so as to reach larger size in a fairly short time.

The results of this study have clearly shown the radial growth characteristics of tropical pioneer and succeeding species. Both the pioneer trees that grow under relatively sunlit conditions throughout their lifecycle have different growth patterns as compared to the tropical succeeding tree species which grow from shaded to sunlight conditions in their lifecycle.

# CONCLUSION

The results reveal that species type and size are important elements in describing the radial growth and cell production of a tree. During the research period, radial growth rate and cell production rate varied with species and tree sizes. Generally, *M. gigantea* and *E. diadenum*, which belong to the pioneer species, have higher radial growth and cell production rate than *D. costulatus*, which is a succeeding species in forest succession. *M. gigantea* and *E. diadenum* with DBH less than 20 cm have

higher cell productions and radial growth rates than those trees with DBH greater than 25 cm. Both the pioneer species grew faster in smaller stem size than larger stem size. In *D. costulatus*, trees with DBH greater than 25 cm have slightly higher cell production and radial growth rate than tree with DBH less than 20 cm. This means this species grows faster during larger stem size than smaller stem size.

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