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Anatomical Characteristics of *Garcinia lucida* (Vesque) and *Scorodophloeus zenkeri* (Harms) Wood and Debarking Response in the South Region Cameroon

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Abstract

The aim of this work was to investigate the possible anatomical changes of *Garcinia lucida* and *Scorodophloeus zenkeri* after the removal of their bark. Debarking was done on individuals of each species at 1.30 m from the soil. The wound was rectangular in shape with 30 cm side. There was a follow-up every three months for nine months during which the survival and rate of regeneration of the bark were recorded. A block of cube was cut from the regenerated and intact wood of species for microtomy and microscopy activities. On the cross-section of each wood, vessel features like density and diameter were measured before and after wounding. Semi-automatic measurements were made using the SpectrumSee digital image analysis software. In the wood of the two species, it appeared that the density of the vessels before debarking was significantly comparable to the density after debarking, while the diameter of vessels in the regenerated wood was smaller. The cambial area increased slightly in the rainy season for all species. After nine months all the species started the restoration of their conductive zone. *G. lucida* heals its wound more rapidly than *S. zenkeri*.

Keywords: NWFP, anatomy, wood, debarking, *Garcinia lucida*, *Scorodophloeus zenkeri*

1. Introduction

Tropical forests are ecosystems which suffer from the depletion of their surfaces and this is directly linked to population growth (Millet, 2001). Thus, the importance of non-wood forest products (NWFP) for local livelihoods and as a means to ensure forest conservation has been widely recognized (Marshall, Schreckenberg, & Newton, 2006). The exploitation of NWFPs has become an increasingly attractive activity for many poor and unemployed people in Central Africa in general and Cameroon in particular (Guedje, Lejoly, Nkongmeneck, & Jonkers). NWFPs are defined as goods of biological origin other than wood, derived from forests, wooded lands and trees outside forests (Food and Agriculture Organization of the United Nation, 1999). About 80% of the people in developing world depend on NWFPs for their primary health and nutritional needs and/or in terms of meeting their subsistence consumption and income needs. Sustainable forest management requires that management ensures the utilization that meets present demand for forest products and services and also ensures that future generations' ability to have the products and services is not compromised. Furthermore the forests are able to regenerate and maintain existing condition (Chamberlain, Bush, & Hammett, 1998). The exploitation of tree species has a variable effect depending on the parts harvested since harvesting of the bark is more damaging in terms of tree survival (Geldenhuys, Syampungani, Meke, & Vermeulen, 2007; Vermeulen, 2009). The bark is produced by a thin layer of cambium cells which surround the xylem and phloem tissues that transport water and nutrients to and from the roots and leaves. The bark also protects plants against fire, fungal and insect attack (Cunningham, 2001). Removal of bark can therefore damage phloem or expose it to desiccation and fungal or parasite attack. This may disrupt the conduction of nutrients and hormones involved in flower bud production for example (Mohr & Schopfer, 1995). The need for resources to repair damage to bark could also result in lower

resource allocation to reproduction. Trees respond differently to harvesting methods, not much information is available on the ecological impacts of bark harvesting (Ticktin, 2004). Bark harvesting interrupts suddenly the water relationship between bark and wood and may affect the water conduction between leaves and roots (Zwieniecki, Melcher, Field, & Holbrook, 2004). As trees consume large amounts of water, they have to develop mechanisms for protection against disturbance of their water balance whereas they should be able to restore the water pathway. Both the diameter and the density of vessels directly influence conductivity (Christensen-Dalsgaard, Fournier, Ennos, & Barfod, 2007; Sellin, Rohejarv, & Rahi, 2008).

In southern Cameroon, *Garcinia lucida* (Clusiaceae) is harvested from undisturbed and generally easy to access forests. Moreover, this species may be endangered by the overexploitation caused by the use of its bark in the production of palm wine. The bark is used as an additive to palm wine production and in distilling fermented palm wine to produce liquor. The bark and the seeds, dried or fresh, are widely used for medicinal purposes to prevent food poisoning and to cure stomach and gynaecological pains, as well as to cure snake bites (Guedje, Tadjouteu, Onana, Nnanga Nga, & Ndoye, 2017). This species is a gregarious species that grows in dense stands on the slopes of forests above 500 meters of altitude. However, there are several sites with a relatively high mortality rate, resulting from an anarchic and intensive exploitation of this resource (Guedje, Zuidema, During, Foahom, & Lejoly, 2007). Studies on the exploitation and management of *Scorodophloeus zenkeri* in the Ngovayang forest in southern Cameroon reveal that trees are generally cut down when diameter at breast height is more than 35cm for the exploitation and marketing of their bark which is used as a condiment or spice (Ndzomo, 2011).

The aim of this paper was to evaluate the short-term responses to wounding in two tree species. We are going to describe first the wood of the two species following IAWA characteristics. Since vessel features can be considered as indicators of anatomical wood reactions following bark harvesting, we should investigate the impact of the wood after nine months by evaluating the features of vessels and other wood elements produced before and after bark harvesting.

2. Material and Methods

2.1 Location of Study Area

The Ngovayang massif, in southern Cameroon, is part of a group of small hills along the Atlantic coast of the Gulf of Guinea. This area is known for its floristic richness and its high level of endemism (Gonmadje, Doumenge, Sunderland, Balinga, & Sonké, 2012). It extends over 102 000 ha between the parallels 3 ° 12'-3 ° 25'N and the meridians 10 ° 30'-10 ° 45'E. The altitude varies between 50 m in the south-western part and 1090 m in the central-eastern part, on the highest peaks. The climate is of the sub-equatorial type (Suchel, 1972). The average annual precipitation is around 2000 mm (Waterloo, Ntonga, Dolman, & Ayangma, 2000). They decrease with increasing distance from the ocean. The average annual temperature is 25 °C (Waterloo et al., 2000). The dense river system is made up of numerous small rivers intersected by falls. Biafrean Atlantic forests rich in Leguminosae-Caesalpinioideae constitute the main type of vegetation encountered in the study area. In addition to global threats such as climate change, destruction of the forest for arable land use and logging have contributed to the destruction of natural vegetation for decades, mainly in low-lying and flat areas that are easily accessible (Letouzey, 1985). Other human pressures, such as hunting or harvesting of forest products, are also notable, even at higher altitudes (Gonmadje et al. 2012).

2.2 Description of Study Species

Garcinia lucida species is a small understory dioecious tree, standing sometimes on stilt roots, reaching 25 - 30 cm in diameter at breast height (dbh) and 12–15 m in height. Trees are of variable aspect, well-branched and evergreen. The stem exhibits yellow and resinous exudates after the dark brown bark is cut or removed. The natural habitat of *G. lucida* is the Atlantic “primary” rain forests. *G. lucida* tree grows in high-density stands covering few hectares with an abundance varying from 348 young matures (5 - 10 cm dbh) to 5 large trees (> 20 cm dbh) per hectare (Guedje et al. 2017).

Scorodophloeus zenkeri (Caesalpiniaceae) is a tree up to 35 m tall and 200 cm in diameter. Its trunk is straight and more or less cylindrical. The base is slightly thickened and has grooves. The top of this tree is open with upright branches. The bark of *Scorodophloeus zenkeri* is gray-yellow, smooth in young individuals then scaly and may have a slice about 1 cm thick, brittle, yellow with a garlic odor (Eyog, Ndoye, Kengue, & Awono, 2006).

2.3 Sampling of Bark Recovery Parameters

For each species, the experimental debarking was carried out on 11 individuals of a diameter class between 10

and 30 cm, during the dry season. These individuals were chosen randomly, five individuals for *S. zenkeri* and six for *G. lucida*. For each species, only trees that had not yet been debarked were selected for the experiment. On each individual, wounds were made at 1 m above ground level. The wound consisted of a rectangular piece of bark 30cm vertically and 10cm width. Six months after, block of wood and bark samples were cut at wound level, in the intact zone and regenerated zone and were immersed in 1/3 ethanol (diluted to 70%), 1/3 glycerin and 1/3 water so as to preserve the intact cambial zone and to ensure the good wood-bark cohesion at least for 72 hours.

Samples sections (transversal, tangential and radial) with a thickness of about 15-30µm were obtained and contain phloem, cambial zone and secondary xylem. We used a sliding microtome (Shenkung Dapples, Mikrot L- serie 1117, Switzerland). The sections were double stained with 0.1% safranin O and fast-green solutions especially good for meristematic tissues (Table 1).

Table 1. Staining and dehydration method

Step number	% ethanol	Other chemicals	time
1	50	/	5 min.
2	/	1g/l aqueous safranin solution	10 min.
3	50	/	2x5 min.
4	75	/	5 min.
5	/	Fast Green, 0.1g in 100 ml 96 % ethanol	4 min.
6	96	/	5 min.
7	100	/	15 min.

Afterwards the sections were mounted immediately on a slide by using Eukitt and fixed with magnets on aluminum foil to be dried in open air. The observations were made under a light microscope of model N-800M. This double staining was carried out in order to be able to differentiate the cell walls in terms of color under a photonic microscope (Dié et al., 2012).

All characters were described using IAWA (International Association of Wood Anatomists) List of Microscopic Features for Hardwood Identification (IAWA Committee, 1989) on transversal, radial and tangential sections.

The following measurements were performed on each individual according to the methodology of (Delvaux, Sinsin, Van Damme, & Beeckman, 2013). In the wood, the density of the vessels in a surface of 1 mm² was calculated, the diameter of the vessels (on a slide, at least 25 vessels were measured), the area occupied by the vessels, fibers, parenchyma and rays were determined. The number of cells constituting the cambial zone was also determined. These measurements were done in the wood before and after debarking, close to the cambial zone.

2.4 Statistical Analysis

All of these semi-automatic measurements were made using the SpectrumSee-F digital image analysis software. Statistical analyzes were carried out using the SPSS software and specifically ANOVA, and the averages were separated by the DUNCAN test.

3. Results

3.1 Characterization of Wood

3.1.1 Anatomical Characteristics of *Garcinia lucida* Wood

The characterization of the wood of a species is the set of processes consisting in determining the anatomical characteristics of the cells that make up the wood.

Growth rings: 1: They are clearly visible and distinct

Vessels: 5: In the vessels, the pores were disseminated or diffuse; Vessels partly solitary, partly in radial multiples of 2-4, or small clusters, absence of features 9, 10, and 11; 13: the type of perforation was simple; 41: the tangential diameter of the vessels was average and varied from 54 to 56 µm (Figure 1a); 45 ': Presence of vessels of two distinct sizes; 48: the vessels were 20-40 per mm^2.

Fiber: 66 ': non-septate fibres present; 67: parenchyma-like fibre bands alternating with ordinary fibres; 70: fibres very thick-walled.

Parenchyma: 76: axial parenchyma diffuse 78: axial parenchyma scanty paratracheal 80: axial parenchyma aliform 82: axial parenchyma winged-aliform 83: axial parenchyma confluent 85: the parenchyma was thicker

than three cells; 86: Axial parenchyma in narrow bands or lines up to three cells wide 92: Four (3-4) cells per parenchyma strand.

Rays: 97: Rays 1-3 cells; 101 ': Absence of false rays; 105: All ray cells upright and / or square 108: Body ray cells procumbent with over 4 rows of upright and / or square marginal cells 109: Rays with procumbent, square and upright cells mixed throughout the ray 115: Rays per millimeter: Number of rays on tangential section per mm: 4-12 (Figure 1b).

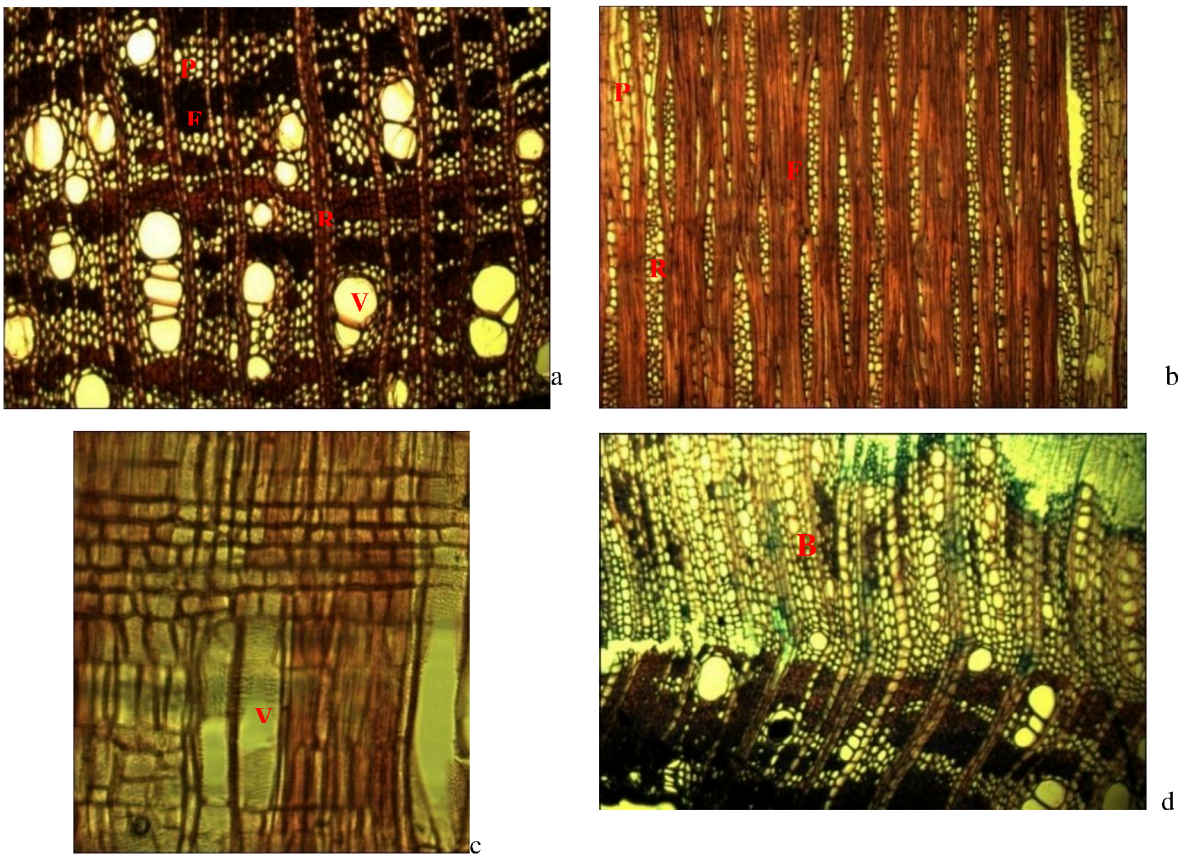

Figure 1. Anatomical sections of *Garcinia lucida* wood (a) transverse section (40x) (b) tangential section (40x) (c) radial section (100x) (d) cross section in regenerated wood (40x); P: parenchyma, F: fibre, R: ray, V: vessel, B: bark

3.1.2 Anatomical Characteristics of *Scorodophloeus zenkeri* Wood

The characteristics observed in the wood anatomy of this species were as follows:
Growth rings: 1: Growth ring boundaries distinct.

Vessels: 5: Wood diffuse-porous; absence of feature 12: Solitary vessel outline circular to oval; 41: The tangential diameter is Medium and varies from 62 to 71 µm (Figure 2a); 45: Presence of vessels of two different sizes; 47: 5 - 20 vessels per square millimetre.

Fibers: 66: Non-septate fibres present 69: Fibres thin- to thick-walled; 70: Fibres very thick-walled.

Parenchyma: 76: Axial parenchyma diffuse 78: Axial parenchyma scanty paratracheal 85: Axial parenchyma bands more than three cells wide; 86: Axial parenchyma in narrow bands or lines up to three cells wide; 92: Four (3-4) cells per parenchyma strand; 93: Eight (5-8) cells per parenchyma strand.

Rays: 97: Ray width 1 to 3 cells; 104: All ray cells procumbent 106: Body ray cells procumbent with one row of upright and / or square marginal cells; 115: Number of rays per mm: 4-12 (Figure 2b); 116: Number of rays per mm >12 /mm.

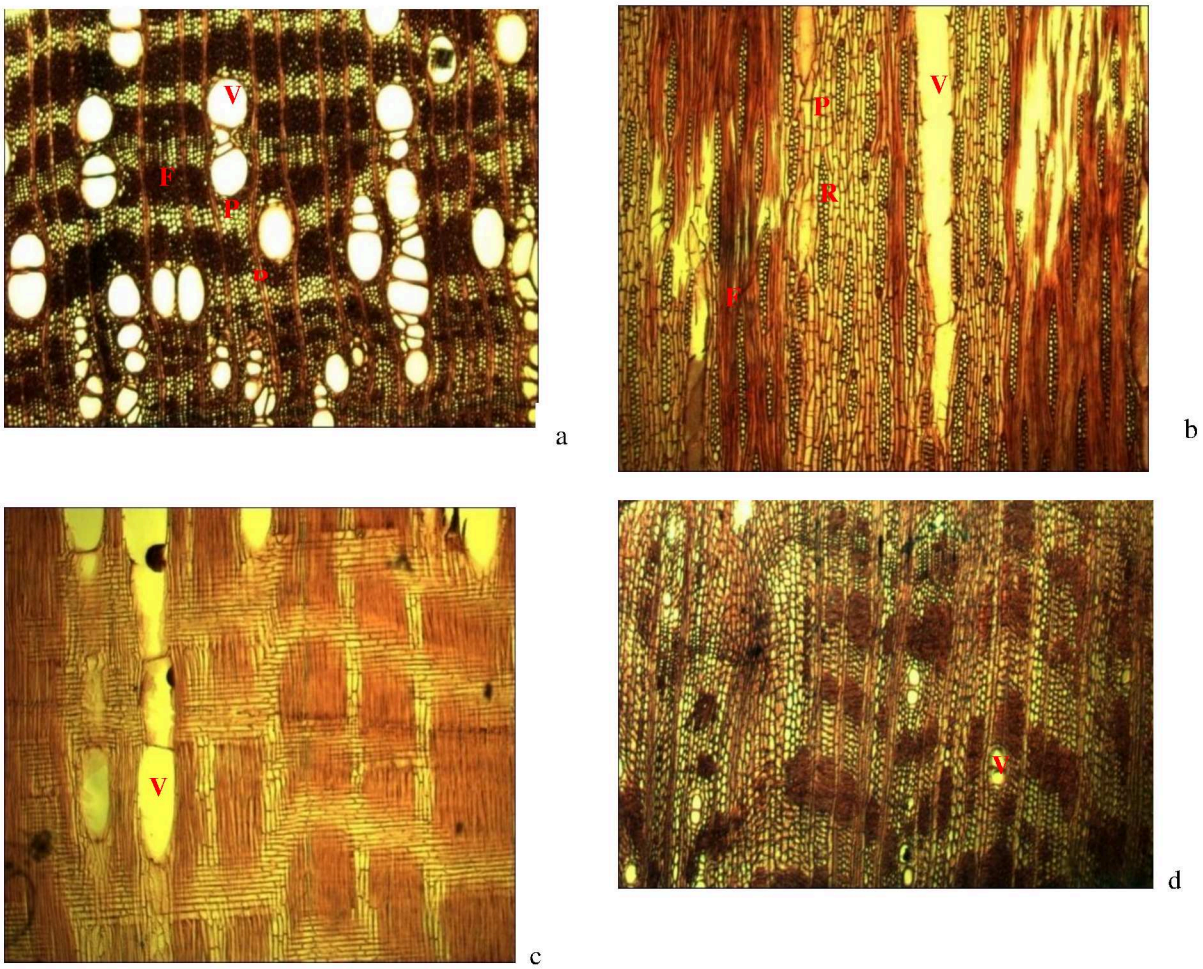

Figure 2. Anatomical sections of *S. zenkeri* wood (a) cross section (b) tangential section (c) radial section (40x) (d) cross section in regenerated wood; P: parenchyma, F: fibre, R: ray, V: vessel

3.2 Recovery of Wound after Debarking

The recovery rate gradually increased in both species, so they are among the species that heal their wound after debarking (table 2, figure 3).

Table 2. Recovery speed of wound as a function of time of the two species

	Recovery speed of wound as a function of time (cm)		
	3 months	6 months	9 months
G. lucida	1.2±0.27	2.4±0.37	3.6±1.29
S. zenkeri	1.05±0.21	1.63±0.91	1.66±0.92

Figure 3. Macroscopic view of bark regeneration on tree stems in the Ngovayang forest a: *G. lucida* at the time of debarking b: *G. lucida* bark regrowth after 9 months c: *S. zenkeri* at the time of debarking d: *S. zenkeri* bark regeneration after 9 months

3.3 Characterization of the Cambial Zone

The cambial zone of these two species was composed of a number of variable cells; and these variations were seasonal. For *Garcinia lucida*, we found that the number of cells constituting the cambial zone in the dry season varies from 06 to 07 cells, whereas in the rainy season it increases and varies from 09 to 10 cells. In the case of *Scorodophloeus zenkeri*, the same observation was made: in the dry season, the cambial zone was composed of 10 to 11 cells, while in the rainy season it consists of 10 to 12 cells.

3.4 Modification in Newly Established Wood

Table 3. Comparison of initial wood and regenerated wood of *G. lucida*

Anatomical Parameters	Wood before debarking	Regenerated wood
Density of vessels (mm²)	33 ± 7.98a	31 ± 13.83a
Diameter of vessels(µm)	56.17 ± 5.33a	44.98 ± 3.52b
Vessel area (µm²)	3369.91 ± 689.82a	2462.69 ± 556.89a
Fibers area (µm²)	9615.70 ± 3140.57b	13718.11 ± 5767.21a
Rays area (µm²)	21158.39 ± 6084.81a	19709.88 ± 3363.36a
Parenchyma area (µm²)	5182.89 ± 1262.39b	11769.74 ± 4796.47a

Means with different letters within the same line are significantly different at $P \leq 0.05$

In *G. lucida*, although the number of vessels per mm² in the wood before debarking (33 ± 7.98 / mm²) and regenerated wood (31± 13.83 / mm²) was different, this density does not differ significantly. With regard to the diameter of the vessels, a significant difference was observed between the initial wood and the regenerated wood. This diameter was higher in the initial wood (56.17± 5.33 µm) compared to the regenerated wood (44.98± 3.52 µm). The areas occupied by parenchyma and fibers were significantly higher in the regenerated wood compared

to the initial wood ($p < 0.05$). However, the surfaces occupied by the vessels and the rays remain statistically comparable ($p > 0.05$) before and after peeling ok bark (table 3).

In addition to these modifications, there were also other changes in the anatomy of this wood. In cross-section, some tissues were still diffuse in the wood; the diameter of the vessels was small and it was not easy to differentiate or clearly distinguish the different tissues of the wood seen in cross section like parenchyma and wood rays (figure 1d).

Table 4. Comparison of initial and regenerated wood of *S. zenkeri*

Anatomical Parameters	Wood before debarking	Regenerated wood
Density of vessels (mm²)	29 ± 18.97a	32 ± 12.29a
Diameter of vessels (µm)	63.43 ± 7.18a	44.39 ± 3.67a
Vessel area (µm²)	14059.59 ± 5066.6a	2256.71 ± 393.52a
Fibers area (µm²)	17338.63 ± 7380.63b	32954.58 ± 18583.63a
Rays area (µm²)	14240.52 ± 2825.51a	12964.09 ± 3784.78a
Parenchyma area (µm²)	6702.11 ± 2451.02b	9036.01 ±3366.72a

Means with different letters within the same line are significantly different at $P \leq 0.05$

The density of the vessels in the regenerated wood was changed from the original wood. Moreover, the diameter of the vessels, the surface occupied by the parenchyma and the rays has also undergone modifications. For *S. zenkeri*, the density of the vessels in the regenerated wood is greater than that of the wood before debarking. It increased from 29 vessels per mm² to 32 vessels per mm² in regenerated wood, but there was no significant difference (table 4). The diameter of the vessels was 63.43 µm for the wood before debarking and 44.39 µm for the regenerated wood; these values are comparable ($p > 0.05$). The area occupied by the rays is comparable in the wood before and after debarking ($p > 0.05$). As for parenchyma and fibers, the area they occupy in regenerated wood was significantly higher than that of the wood before bark peeling ($p < 0.05$).

There were also other changes in the anatomy of this wood. In regenerated wood, some tissues were not yet well differentiated, including parenchyma, rays and fibers. It was also observed that the diameters of vessels were very small compared to vessels before debarking (figure 2d).

4. Discussion

The tree has defence mechanisms based on its ability to compartmentalize wounds. But the effectiveness of these mechanisms is limited in the case of excessive injuries or too severe pruning. Among the practices that result in tree weakening, cuts on large sections and debarking can be cited as an example. The closure of the wound begins with the formation of callus tissue consisting of soft tissue masses of the parenchyma (Fahn, 1985), that gradually invade the wound surface (Bai, Chaney, & Qi, 2005). When the wounds are too numerous and too large, the wood becomes vulnerable and is inevitably infected and colonized by the lignivorous fungi which slowly decompose it. The response of the tree to debarking is first of all specific to each species (Delvaux, Sinsin, Darchambeau, & Van Damme, 2009). After the tree bark was removed from the field, no deaths from the 11 sampled trees were recorded. This could be explained by the debarking on a rectangular area of 10cm wide by 30cm long. This is not a 100% debarking which in fact consists in removing the bark over the entire circumference of the tree, because when debarking is done at 100% it prevents the growth of the tree, because the wound is considerable and damages the tissues of the nutrient pipes of the tree, namely the phloem for nutrients and the xylem for water (Delvaux et al., 2009).

The results obtained in the characterization of the wood were similar to those of the INSIDE WOOD database concerning those species. Changes were observed in the cambial zone of these two species and this according to the seasons. The cambial zone is the part of the tree that is found between the primary xylem and the primary phloem. In the cambial area of the wood, there is an area of cells with little differentiation to active divisions. This generating zone called vascular cambium produces cells that differentiate into secondary xylem and secondary phloem or liber (Dié et al., 2012). In the cambial zone of *G. lucida* and *S. zenkeri*, during the rainy season there was an increase in the cells constituting this zone. This could be explained by the favorable climate conditions in this season and the abundance of rainfall. Some studies of the causes of anatomical changes in water supply show that even a short drought during the period of timber formation would result in a corresponding reduction in auxin content, which is a phytohormone inducing cell division (Polge & Keller, 1986). In fact, parenchyma is the most abundant tissue in the regenerated wood; they are made up of globular shaped cells and participate in the nutritive functions of the tree (Evert, 2006).

For the two species studied, regenerated wood vessels were smaller. As regards the density of the vessels, in *G. lucida*, it is slightly higher in the initial wood than in the regenerated wood. In the wood of *S. zenkeri*, it is found that the density of the vessels in the regenerated wood is slightly greater than that of the wood before debarking. Similarly, the diameter of the vessels and the area occupied by the vessels in the regenerated wood are smaller than those found in the original wood. Unlike *G. lucida*, the results obtained in *S. zenkeri* wood are also similar to those of Delvaux, Sinsin, Van Damme, & Beeckman (2010) who found that during early wound healing, all the species they studied produced vessels with smaller area than in unaffected wood and this significantly decreased the specific conductive area in eight of the investigated species. Small vessels contribute to a safer conducting system and act as adaptive mechanisms to protect the tree (Aloni & Zimmermann, 1983). One of the consequences of the removal of the bark is the obstruction of the conductive vessels which cause the accumulation of auxin. This phenomenon results to increase of differentiation rate of the vessels in the regenerated wood which would lead to more numerous and narrower vessels in this wood (Mwange, Hou, & Cui, 2003; Evert, 2006). In addition, Mwange et al. (2003) also stated that the first steps bark recovery, namely division and differentiation of non-mature cells and the formation of cambium, are dependent on auxin.

5. Conclusion

In conclusion, *Garcinia lucida* and *Scorodophloeus zenkeri* changes in space and time in the anatomy of their wood after wounding; structural changes in the newly established wood are observed, in particular the tissues of regenerated wood such as rays and vessels which are not yet well differentiated as in the wood before wounding; *G. lucida* regenerates its bark more rapidly than *S. zenkeri*. In *G. lucida*, the number of vessels per mm² in the wood before debarking (33 ± 7.98 / mm²) and regenerated wood (31± 13.83 / mm²) is different. With regard to the diameter of the vessels, a significant difference is observed between the wood before (56.17± 5.33 µm) and after debarking (44.98± 3.52 µm). The areas occupied by parenchyma and fibers are significantly higher in the regenerated wood compared to the initial wood ($p < 0.05$). For *S. zenkeri*, the density of the vessels has increased from 29 vessels per mm² to 32 vessels per mm² in regenerated wood, the diameter of the vessels is 63.43 µm for the wood before debarking and 44.39 µm for the regenerated wood. As concerning *S. zenkeri*, the area occupy by the parenchyma has increased after wounding. For these two species, smaller vessels obtained in regenerated wood contribute to a safer water-conducting system and are an adaptive mechanism. So, vessel diameter is an important parameter for assessing the ascension of water and minerals from roots to leaves and the adaptation of plants to their environment. We can conclude with Delvaux et al. (2010) that vessels appeared to be very good anatomical indicators of the tree's reactions to debarking.

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