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ORIGINAL PAPER

Wound reaction after bark harvesting: microscopic and macroscopic phenomena in ten medicinal tree species (Benin)

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Abstract In Africa, little is known about how the vascular anatomy of medicinal tree species is influenced by bark harvesting, and the ability of species to react against debarking needs to be better understood. This study aims to evaluate the temporal and spatial impact of bark harvesting on wood anatomy and to determine the extent to which a tree's ability to close the wound after bark harvesting is affected by anatomical changes in the wood. We harvested bark from ten medicinal tree species located in an *Isoberlinia doka* woodland in Central Benin. Two years after debarking, the wound closure was measured and one tree per species was cut at the wound level to collect a stem disc. On the cross section of each disc, vessel features (area, density and specific conductive area) were measured in the radial direction (before and after wounding) and on three locations around the disc surface. We found that during early wound healing, all species produced vessels with a smaller area than in unaffected wood and this significantly decreased the specific conductive area in eight of the investigated species. However, after 2 years, only six trees had restored their specific conductive area. In addition, a significant positive correlation ($r = 0.64$, $P < 0.005$) confirmed the relationship between the specific conductive area and tissue production to close the wound and delineated the study group into two groups of trees. Therefore, we concluded that vessels appeared to be very good anatomical indicators of the tree's reactions to debarking.

Keywords Bark harvesting · Specific conductive area · Re-growth dynamics · Vessel features · Wood anatomy

Introduction

In African countries, 80% of the population uses medicinal plants for health care on a regular basis. The latter includes the use of medicinal tree barks. Such bark is often purchased at local, regional or international markets. Bark harvesting frequently results in a substantial wounding of the tree, particularly when the bark is completely removed, down to the wood layer, and without concern for subsequent tree survival. Besides the harvesting intensity, a species' vulnerability to bark harvesting depends on its capacity to recover from bark stripping (Geldenhuys and Williams 2006). Since 2003, several bark recovery studies have been carried out in a number of countries: Zambia, Malawi (Geldenhuys and Williams 2006; Syampungani 2006; Geldenhuys et al. 2007), South Africa (Geldenhuys 2004; Vermeulen and Geldenhuys 2004) and Benin (Delvaux et al. 2009). Overall, 33 species (12 in Benin, 12 in South Africa, 10 in Malawi and 5 in Zambia; some species were studied in more than one country from different woodland and forest areas) were studied to develop an understanding of species-specific responses to bark harvesting. Where it occurs, bark recovery takes place

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through the development of new tissues from the wound edges and/or from its surface. For instance, debarked *Prunus africana* rapidly produced bark through cambium development on the wound surface (Cunningham and Mbenkum 1993; Vermeulen and Geldenhuys 2004; Syampungani 2006; Geldenhuys et al. 2007), but no information was given to determine whether re-growth was from a new or pre-existing cambium. *Ocotea bullata*, *Ilex mitis*, *Albizia adianthifolia* (Syampungani 2006; Vermeulen 2006; Geldenhuys et al. 2007), *Khaya senegalensis* and *Lannea kerstingii* (Delvaux et al. 2009) showed an effective recovery from the edges of the wound. At the same time, some species showed no or only poor recovery and were thus not able to close the wound: *Afzelia africana*, *Burkea africana*, *Lophira lanceolata* (Delvaux et al. 2009), *Cryptocarya myrtifolia*, *Elaeodendron transvaalense*, *Julbernardia globiflora*, *Xymalos monospora* and *Zanthoxylum davyi* (Syampungani 2006; Geldenhuys et al. 2007).

The macroscopic phenomenon of bark recovery observed through these studies is an expression of tissue modification reactions to injury at the microscopic level. Indeed, bark harvesting suddenly interrupts the water relation between bark and wood and may affect the water conduction between leaves and roots (Zwieniecki et al. 2004). As trees consume large amounts of water, they have to develop mechanisms to protect against the disruption of their water balance and be able to restore the water pathway. According to the modified Hagen–Poiseuille equation (Reyes-Santamaria et al. 2002), hydraulic conductivity is proportional both to vessel radius (fourth power) and vessel density. Therefore, both the diameter and the density of vessels directly influence conductivity (e.g., Lovisolo and Schubert 1998; Reyes-Santamaria et al. 2002; Christensen-Dalsgaard et al. 2007; Sellin et al. 2008). Many research efforts focused on the short-term responses to wounding (a few days up to a few months) as expressed by compartmentalization, wound callus formation, wound reaction of the parenchyma cells and lignin distribution in the xylem, etc. (e.g., Shigo 1986; Schmitt and Liese 1993; Stobbe et al. 2002; Frankenstein et al. 2006). However, bark restoration in the context of sustainable harvesting of medicinal tree species needs to be studied over a longer period. Indeed, a 2-year survey would be the minimum period required to gain reliable information on a wounded tree's survival and to analyze the mechanisms of change in vessel features following wounding. So far, vascular system response to mechanical injury has only been studied using species from temperate regions (e.g., Li et al. 1982; Rademacher et al. 1984; Shigo 1986; Li and Cui 1988; Schmitt and Liese 1990; Schmitt and Liese 1993; Novitskaya 1998; Stobbe et al. 2002; Mwange et al. 2003; Dujesiefken et al. 2005; Frankenstein et al. 2005; Frankenstein et al. 2006) rather than in tropical regions (Lev-Yadun and Aloni 1993; Thomas et al. 1995; Christensen-Dalsgaard et al. 2007). Among the 20 tree species already studied, only 1 was a medicinal tree, i.e., *Eucommia ulmoides* (Li et al. 1982; Li and Cui 1988). For African medicinal trees, with the exception of the species mentioned by Noel (1970), little is known about how vascular anatomy is influenced by bark harvesting and the impact this might have on re-growth. Previous research projects focused either on relatively small harvested surface areas of bark, sometimes only a few cm^2 (Rademacher et al. 1984; Lev-Yadun and Aloni 1992; Stobbe et al. 2002; Frankenstein et al. 2005), or on total girdling, whereby all bark was removed from the trunk over a height of 1–2 m (Li et al. 1982; Li and Cui 1988). This contrasts with our study, where the harvested proportion of bark represented 20–50% of the circumference of the tree to mimic traditional healer practices in Benin.

The aim of this paper was to study the anatomical micro-reactions to wounding in ten medicinal tree species from Benin known to differ widely in their potential bark recovery (Delvaux et al. 2009). Xylem anatomical features have the advantage of permanently "archiving" the developmental mechanisms in response to environmental changes, which can be evaluated retrospectively (e.g., Sass and Eckstein 1995). Xylem vessel features can thus be considered as indicators of anatomical wood reactions following bark harvesting. We felt that it was essential to calculate the temporal and spatial impact of bark harvesting on both vessel density and area, and specific conducting area which is the sum of vessel areas per unit area. First, we investigated the impact of the bark harvesting on the wood formation over 2 years, both at the wound edge and around the trunk. Second, we hypothesized that the ability to close the wound after bark harvesting depends on wood anatomical characteristics of the new wood. To test this hypothesis (1) vessel features were compared in the wood produced before and after bark harvesting; and (2) the ability to return to the normal conductive area over a 2-year period, expressed in percentage of conductive area recovery, was related to the percentage of wound closure over the same period.

Materials and methods

Study area

The study was carried out in the forest reserve Forêt Classée des Monts Kouffé (8°30′–8°52′N, 1°40′–2°27′E) situated approximately 400 km north of Cotonou. This is one of the largest protected areas in the country. It covers 180,300 ha composed of woodlands, dry forests, savannas and gallery forests, and is located in the Sudano-Guinean region (Adomou et al. 2007). Study sites were selected in an *Isoberlinia doka* woodland on ferruginous soils. Like most protected areas in Benin, the Forêt Classée des Monts Kouffé is somewhat degraded due particularly to the encroachment of agriculture. Our trees were harvested away from farms. The tropical rainy season between May and October has a unimodal regime. Mean monthly rainfalls during the study period in 2004, 2005 and 2006 were 138, 189 and 165 mm respectively (ranging from 21.5 to 306.2 mm). The annual temperature ranged from 25 to 34°C and were similar for each year of the study. Rainfall and temperature data were supplied by *Agence pour la Sécurité de la Navigation Aérienne en Afrique et à Madagascar* (ASECNA) in Benin.

Study species and sample collection

As a first step, a large number of medicinal tree species were selected to compare a sufficient diversity of responses to bark harvesting. Several ethnobotanical interviews were held with traditional healers and other members of the local populations to learn their preferences for tree species used for health care (Bockx 2004). Subsequently, ten of the most frequently used species were chosen for the study (Table 1). In November 2004, the bark was removed from the sample trees with a diameter at breast height (d.b.h.) that ranged from 10 to 20 cm (Table 1). As we were working in a forest reserve and used a destructive technique (trees needed to be cut), we decided to use only one tree from ten selected species. Wounds were made 1 m aboveground level. The wound consisted of a rectangular piece of bark 30 cm vertically and the lateral extent of the wound varied between 9 and 22.7 cm width, i.e., width as percentage of trunk circumference (Table 1). In November 2006, trees were cut at wound level and a wood disc was collected from the middle of the wound. After sampling, discs were immediately stored in FAA (formaldehyde–acetic acid–ethanol). Wood disc samples were deposited in the wood collection of the Royal Museum for Central Africa, Tervuren, Belgium (for accession numbers, see Table 1).

Sample preparation and wood anatomical measurements

Disc samples were embedded in PEG 2000 (PolyEthylene Glycol 2000) to keep bark and wood together. They were then sanded using a series of sandpapers with grain size varying from 50 to 1,200. On the cross section of the discs, vessel features were measured along eight radii (Fig. 1). These radii were chosen on specific locations: (1) two radii on both the left and the right sides of the wound, at the point where bark harvesting stopped and where the radial growth started to close the wound (W); (2) one radius on both the left and the right sides of the wound and at 2 cm from the wound (F); and (3) two radii at the opposite side of the wound on the disc sample (O). Along each radius, 11 quadrates each with an area of 3 mm^2 (rectangle of 2,000 μm on the tangential direction and 1,500 μm on the radial direction) were measured in intact wood before the wound. According to the quantity of wood that was tangentially produced during the 2-year period after bark harvesting, one to nine quadrates were measured on the same radius in wood produced after the wound was inflicted. Vessel density (number of vessels per mm^2) and vessel area (lumen area in μm^2) were measured manually making use of digital image analysis software AnalySIS 3.2 (Soft Imaging System GmbH, Münster, Germany), at an optical magnification of ten. Specific conductive area represents the percentage of cross-sectional area occupied by vessels per unit xylem area and was calculated for each quadrate.

Table 1 Medicinal tree species with their respective accession number, from Tervuren Wood (TW-sample stem disc), diameter (d.h.b.) and width of wound after bark harvesting

Species	Family	TW number	d.b.h. (cm)	Wound width cm (% of circumference)
Afzelia africana Sm.	Fabaceae (C)	58888	18.0	9.0 (20%)
Burkea africana Hook.	Fabaceae (C)	58887	13.3	19.0 (50%)
Detarium microcarpum Guill. & Perr.	Fabaceae (C)	58883	18.0	8.7 (20%)
Khaya senegalensis (Desv.) A. Juss.	Meliaceae	58882	14.7	22.7 (50%)
Lophira lanceolata Van Tiegh. ex Keay	Ochnaceae	58889	14.1	16.5 (50%)
Mangifera indica L.	Anacardiaceae	58886	17.0	20.0 (50%)
Maranthes polyandra (Benth.) Prance	Chrysobalanaceae	58885	16.0	9.0 (20%)
Pseudocedrela kotschyi (Schweinf.) Harms	Meliaceae	58884	18.0	11.2 (20%)
Pterocarpus erinaceus Poir.	Fabaceae (P)	58880	15.0	10.3 (20%)
Uapaca togoensis Pax	Euphorbiaceae	58881	15.5	9.0 (20%)

C Caesalpinioideae, *P* Papilionoideae

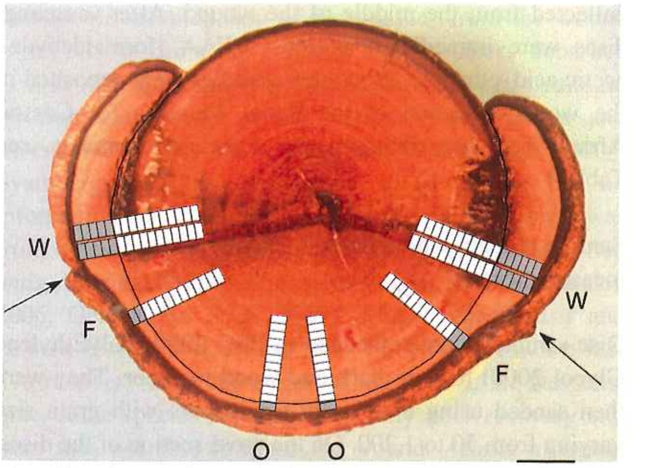

Fig. 1 Wood anatomical measurements were made along eight radii on the disc sample. Four radii on the wound: *W*; two radii at 2 cm from the wound: *F*; and two radii at the opposite side of the wound: *O*. The eight radial strips comprised 11 quadrates in intact wood before bark harvesting (*white*) and various numbers of quadrates after wounding (*gray*). The number of quadrates is linked to the diameter growth after a 2-year period following harvesting and thus depends on the species type. The *dark line* shows the position of cambium at the time of wounding. *Arrows* indicate the wound limit. *Scale bar* 2 cm. Specimen number TW 58882, part of the Tervuren Wood collection

Wound closure measurements

Depending on the species, recovery starts from different spots situated on the whole wound surface (=sheet growth) and/or from the wound side (=edge growth). Two years after bark harvesting, a tracing paper was used to find limits of sheet growth on the wound and to determine its surface area. Thus, this surface area was calculated using the APS ASSEESS program. We measured edge growth surface area on both the left and the right sides of the wound. For calculating the total recovery area, sheet growth and edge growth (left + right sides) areas were summed. The results were expressed as a percentage of debarked area (i.e., area of re-growth against total area debarked).

Statistical analyses

To study the influence of bark harvesting on wood anatomy, two repeated measures analyses GLM were carried out in Systat 11. These analyses were confined to the place of wounding (W). Thus, data from the four radii on the wound were gathered to measure: (1) the impact of the wound on vessel features (density, size) immediately after bark harvesting, through a comparison of the 11 quadrates in intact wood produced before bark harvesting to the first quadrate grown after wounding (A1); and (2) wound impact after a period of 2 years, which was done by comparing the 11 quadrates in intact wood produced before bark harvesting to the last quadrate grown 2 years after wounding (A2). The null hypothesis tested was that there was no difference between vessel features before and after wounding. We tested using the hypothesis test, a C-matrix. To calculate the spatio-temporal influence of bark harvesting at complete trunk level, we used a two-way ANOVA (Statistica 6.0) comparing three different places on the wood disc (at wound level, 2 cm from the wound and at the opposite side of the wound) at three different time periods [the quadrate just before wounding (B), the quadrate just after wounding (A1) and the last quadrate produced by the tree after a period of 2 years (A2)]. Post hoc comparisons between group averages were made with a Tukey's HSD test. Finally, to evaluate the relation between the percentage of specific conductive area recovery $\left(\frac{\text{Conductive area after wounding}}{\text{Conductive area before wounding}} \times 100\right)$ and the percentage of wound closure after a 2-year period, a Pearson correlation coefficient (Statistica 6.0) was calculated for the ten samples exclusively on the wound (W).

As we have one tree for each species, our results will be interpreted only to understand the anatomical mechanism affecting bark recovery. It is not our intention to study the "species effect" of this mechanism.

Results

Variation before and during the early stage of wound healing

The repeated measures analysis GLM showed that there was an effect of bark harvesting on vessel density just after wounding. Five species produced higher vessel density, whereas five other species had lower vessel density than before wounding (Fig. 2a). However, this change in vessel density was significantly higher only in the case of *D. microcarpum* and *P. erinaceus*, whereas it was significantly lower only for *K. senegalensis* and *B. africana*. Vessel area showed a similar pattern for all ten species studied: vessels became significantly smaller (Fig. 2b). A lower specific conductive area was found for nine species whereby this modification was shown to be significant for eight out of nine studied species (Fig. 2c). Nevertheless, only *D. microcarpum* showed a higher specific conductive area, although this difference was not significant. Mean values for vessel density and vessel area values measured in intact wood can be used to characterize each species (Table 2). During the early stage of wound healing, *K. senegalensis* developed callus without vessel production (Fig. 2). Moreover, it was noticed that as a first reaction after wounding, *D. microcarpum* developed traumatic canals that were exceptionally large, very close to each other and observed to occur on the whole

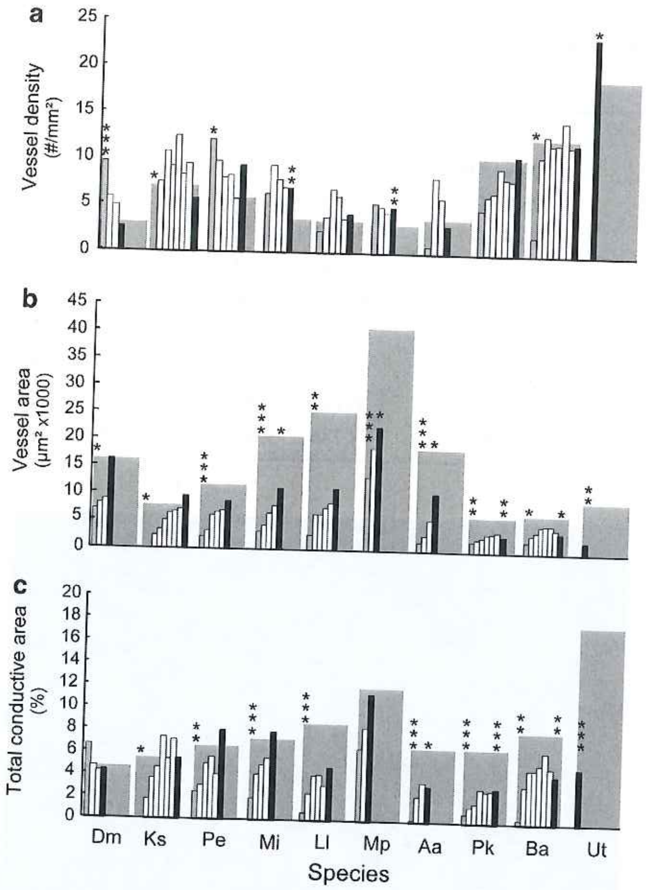

Fig. 2 Repeated measures GLMs of vessel density (#/mm^2) (**a**), vessel area (µm^2) (**b**) and conductive area (%) (**c**) used to test the impact of bark harvesting on vessel features before and after wounding. The different bars represent the consecutive wood quadrates (3 mm^2) produced after wounding. The *broad dark gray bar* represents the average value of the 11 quadrates for each of the four radii in the intact wood before bark harvesting. The *light gray bar* represents the first quadrate produced after wounding. The number of *white bars* depends on the radial growth rate of each species, i.e., the faster the growth the more are the bars. The *black bar* represents the last quadrate produced 2 years after wounding. Transformation types: log (vessel density), sqrt (vessel area) and arcsin (conductive area). Significance levels for differences between values for *white or black bars* (after wounding) and *gray bars* (before wounding): *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Aa, *Afzelia africana*; Ba, *Burkea africana*; Dm, *Detarium microcarpum*; Ks, *Khaya senegalensis*; Lk, *Lannea kerstingii*; Ll, *Lophira lanceolata*; Mi, *Mangifera indica*; Mp, *Maranthes polyandra*; Pb, *Parkia biglobosa*; Pe, *Pterocarpus erinaceus*; Pk, *Pseudocedrela kotschyi*; Ut, *Uapaca togoensis*

trunk circumference (Fig. 3). With *M. polyandra*, tyloses induced by bark harvesting were observed in vessels near the wound limit (Fig. 4).

Diminishing effect of wounding with time

Over a 2-year period following debarking, a significant trend toward normal vessel density was observed for *A. africana*, *B. africana*, *D. microcarpum*, *K. senegalensis*, *L. lanceolata*, *P. kotschyi* and *P. erinaceus* (Fig. 2a). Only four species (*D. microcarpum*, *K. senegalensis*, *L. lanceolata* and *P. erinaceus*) produced vessels with the same area as that before wounding (Fig. 2b). As was the case with vessel density, specific conductive area returned to normal for *D. microcarpum*, *K. senegalensis*, *L. lanceolata*, *M. indica*, *M. polyandra* and *P. erinaceus* (Fig. 2c). The four other species exhibited a significantly lower conductive area than that before wounding.

Spatial diminishing effect of wounding on conductive area

Using a two-way ANOVA (Fig. 5), we found that the specific conductive area measured after bark harvesting was disturbed at various distances from the wound and sometimes up to 2 years after bark harvesting. However, the wood anatomy of *M. indica* and *P. erinaceus* changed very little in time and space. A significant reduction of the conductive area was observed only as a first reaction after wounding. Two years later and at 2 cm away from the wound, the initial conductive area was restored. After the same period, wound influence on wood anatomy was still in evidence locally for *A. africana*, *B. africana*, *P. kotschyi* and *U. togoensis*. At 2 cm away from the wound, the specific conductive area of these species was no longer affected. The conductive area of *L. lanceolata* was significantly lower up to distances of 2 cm from the wound and this situation remained during the 2 years of observation. Just after wounding and 2 years later, at the place where the wound was inflicted and at the opposite side on the disc, *D. microcarpum* and *M. polyandra* showed the same conductive area values. *K. senegalensis* presented a specific characteristic as the impact of bark harvesting was not limited to the wound level but extended to the opposite side of the stem disc. Indeed, at this location, its specific conductive area was significantly higher than before bark harvesting, while on the wound it was significantly lower. Over a 2-year period following harvesting, the effect of the wound on this species' reaction diminished to the point where it was no longer of significance.

Relationship between micro and macro levels

Macroscopically measured, a species' wound closure was significantly correlated with the ability to return to its normal conductive area over a 2-year period after bark harvesting (Pearson correlation coefficient, $r = 0.64$, $P < 0.005$). Following this test, two groups clearly appeared (Fig. 6). In group A, individuals of *D. microcarpum*, *K. senegalensis*, *M. polyandra*, *M. indica* and *P. erinaceus* not only presented a good percentage of wound closure (46–77.1%), but also had a good recovery of conductive area (75–113%) (see

Table 2 Results obtained for vessel density and vessel area in the eight rays in intact wood (before bark harvesting) for ten medicinal tree species

Species	Vessel density (#/mm^2)		Vessel area (μm^2)	
	Mean ± SD	Range	Mean ± SD	Range
A. africana	3.6 ± 0.4	3.2–4.1	17,129 ± 8,861	839–22,499
B. africana	12.2 ± 1.1	10.8–13.6	7,121 ± 3,714	485–49,526
D. microcarpum	2.8 ± 0.3	2.4–3.2	15,880 ± 10,090	485–49,526
K. senegalensis	6.7 ± 0.6	5.6–7.3	8,910 ± 5,649	347–55,702
L. lanceolata	3.2 ± 0.3	2.8–3.8	24,790 ± 11,815	2,341–63,641
M. indica	3.6 ± 0.2	3.2–3.8	20,296 ± 9,906	1,455–62,747
M. polyandra	2.7 ± 0.5	2.0–3.2	37,061 ± 17,026	2,325–98,214
P. erinaceus	5.8 ± 0.5	5.3–6.6	10,355 ± 5,669	362–32,109
P. kotschyi	9.4 ± 1.4	6.5–10.5	6,154 ± 3,007	716–26,149
U. togoensis	20.4 ± 1.5	18.9–22.6	8,786 ± 3,964	508–26,580

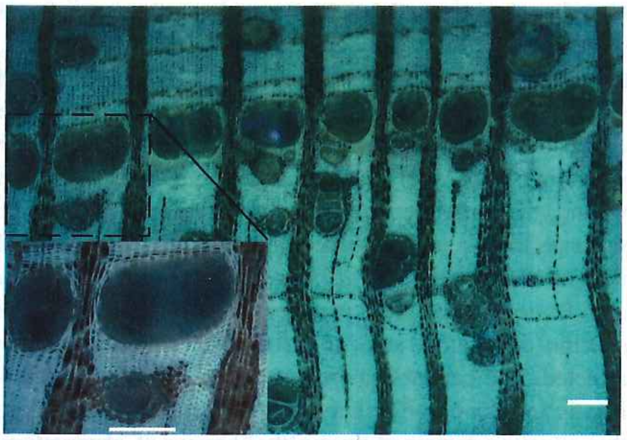

Fig. 3 Fluorescence microscopy of traumatic canals in secondary xylem of *D. microcarpum* on transverse section of stem disc. Formation of canals was induced by bark harvesting on the whole trunk circumference. *Scale bar* 200 μm

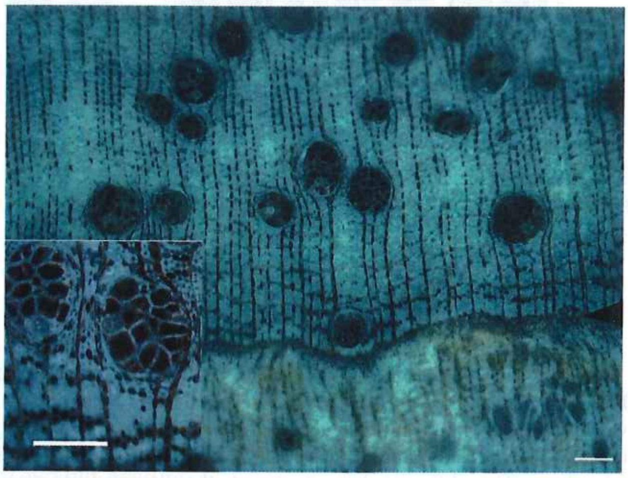

Fig. 4 Fluorescence microscopy of tyloses in *M. polyandra*. Vessels completely filled with tyloses on transverse section of stem disc. *White arrow* position of cambium at the time of wounding. *Scale bar* 200 μm

Fig. 7 for *K. senegalensis* and *M. polyandra*). *D. microcarpum* (113%) was the only species that actually showed a higher specific conductive area than that before wounding. In group B, individuals of *A. africana*, *B. africana*, *L. lanceolata*, *P. kotschyi* and *U. togoensis* presented opposite characteristics: weak wound closure (0–21.9%) and weak recovery of conductive area [37–60 (74)%] (see Fig. 7 for *U. togoensis*). The specimen of *L. lanceolata* did not follow our hypothesis, since, despite achieving a normal conductive area, the tree did not produce wood to close the wound. Indeed, this specimen showed only limited re-growth in the radial direction and not tangentially.

Discussion

Anatomical changes during early stage of wood healing

During the early stage after wounding and for the ten species studied, similar anatomical changes were observed: i.e., occurrence of a lower vessel area and lower specific conductive area. This finding corresponds to earlier results obtained on *Acer saccharum*, *Betula alleghaniensis*, *Fagus grandifolia* (Rademacher et al. 1984), *Acer rubrum* (Aloni and Zimmermann 1984), *Melia azedarach* (Lev-Yadun and Aloni 1993), *Betula pendula* (Novitskaya 1998), *Tilia* spp. (Stobbe et al. 2002) and *Populus tremula* × *Populus tremuloides* (Frankenstein et al. 2005; Frankenstein and Schmitt 2006). In our study, five out of the ten trees showed a tendency toward increasing vessel density immediately after wounding. Nevertheless, since the vessels that were formed after injury were also significantly smaller, all ten species significantly reduced their specific conductive area. Smaller vessels contribute to a safer water-conducting system and are an adaptive mechanism to protect trees against external stresses (Aloni and Zimmermann 1984; Shigo 1984; Verheyden et al. 2005). Bark harvesting results in an obstruction to auxin flow, which leads to localized

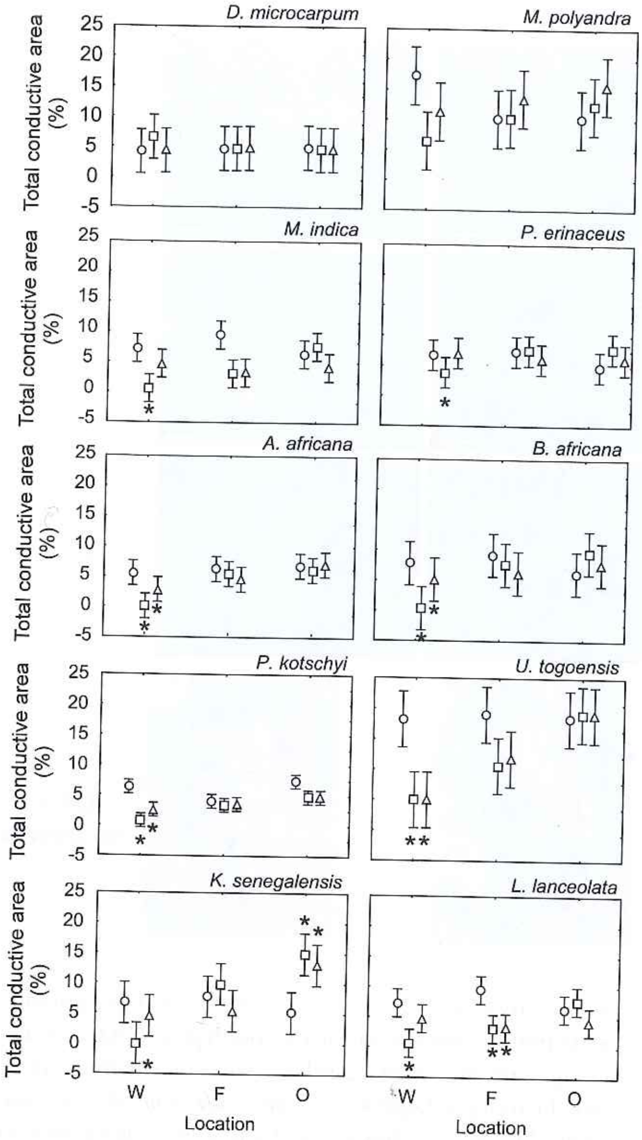

Fig. 5 Two-way ANOVA of conductive area from ten medicinal tree species were used to test the impact of wounding at three different positions on the stem disc: on the wound (*W*); at 2 cm from the wound (*F*); at the opposite side of the wound (*O*). At three different times: before the wound (*circle*), during the early stage of wound healing (*square*) and 2 years after bark harvesting (*triangle*). $^*P < 0.05$

auxin accumulation (Aloni and Zimmermann 1984), inducing an increase in the rate of vessel differentiation, thus resulting in more numerous but narrower vessels (Aloni and Zimmermann 1984; Aloni 1992; Mwange et al. 2003; Evert 2006; Frankenstein and Schmitt 2006). Mwange et al. (2003) have also stated that the first steps in bark recovery (callus initiation, division and dedifferentiation of immature xylem cells, cambium formation) are auxin dependent. However, the first reaction that a tree starts to protect itself after bark harvesting is producing a callus directly in contact with the wound. The latter callus is formed from undifferentiated xylem cells at the stage of primary wall formation. It exclusively consists of parenchymatous tissue without vessels, fibers or ray structures (Stobbe et al. 2002). If the callus was extensive as in *A. africana*, *B. africana* and *K. senegalensis*, almost no vessels were produced in the immediate vicinity of the wound (Fig. 2). Frankenstein et al. (2005) described how initially the formation of large, thin-walled parenchymatous cells appeared along the wound edge and thus two different strategies of callus formation developed during the first 70 days after wounding in *Populus tremula* × *Populus tremuloides*: (1) formation of a wound cambium within the parenchymatous zone as a tangential extension of the undisturbed cambium, or (2) formation of a wound cambium through dedifferentiation of mature secondary phloem cells followed by re-differentiation into a cambial tissue. As we did not observe any production of xylem and phloem cells around a portion of the included phloem, we may assume that our ten species follow the first strategy to close the wound from the edge.

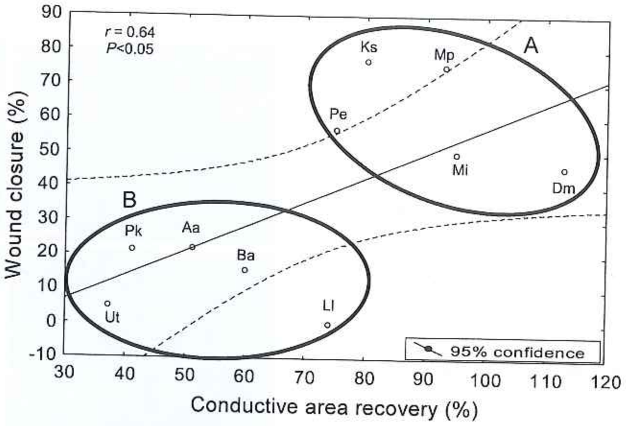

Fig. 6 Correlation (Pearson coefficient, $r = 0.64$, $P < 0.05$) between percentage of wound closure and percentage of recovery of conductive area. Two groups appear: group A where species present a good percentage of wound closure (>40%) and of recovery of conductive area (>70%); and group B where species display a weak percentage of wound closure (<30%) and of recovery of conductive area (<60%, except *L. lanceolata*). Aa, *Afzelia africana*; Ba, *Burkea africana*; Dm, *Detarium microcarpum*; Ks, *Khaya senegalensis*; Ll, *Lophira lanceolata*; Mi, *Mangifera indica*; Mp, *Maranthes polyandra*; Pe, *Pterocarpus erinaceus*; Pk, *Pseudocedrela kotschyi*; Ut, *Uapaca togoensis*

Finally, the tyloses observed in vessels of *M. polyandra* are the materialization of a well-known phenomenon, the compartmentalization (Sun et al. 2006). This phenomenon plays a key role in the defense of a tree after wounding. Tyloses are outgrowths of parenchyma cells into the lumen of vessels through pits (Evert 2006). Their formation is a common response to traumas such as bark harvesting and they prevent the spread of pathogens throughout the plant via the plugging of xylem vessels (Clerivet et al. 2000).

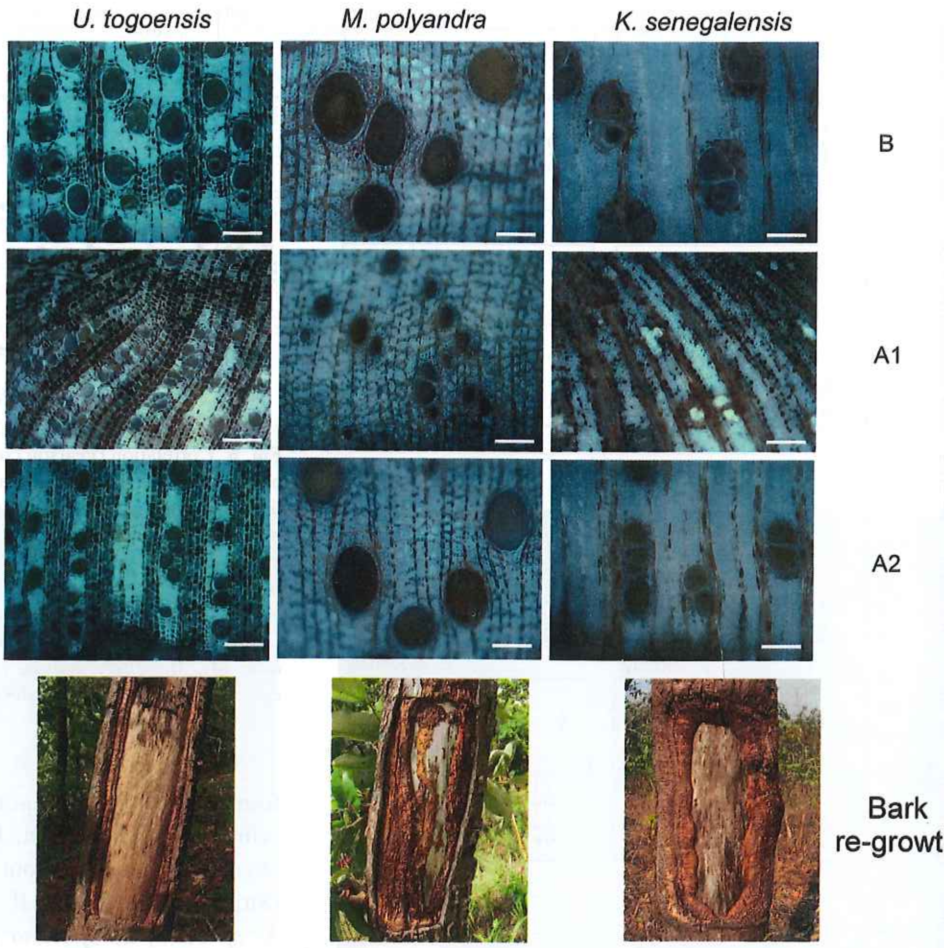

Fig. 7 Fluorescence microscopy of vessel features (density and size) on transverse section of stem discs and macroscopic view of bark regeneration on tree stems in the Forêt Classée des Monts Kouffé (Benin). Vessels before bark harvesting (*B*), at early stage of wood healing (*A1*) and after a period of 2 years (*A2*). Bark: edge growth for *U. togoensis* and *K. senegalensis* and sheet growth for *M. polyandra*. *Scale bar* 200 μm

Diminishing bark harvesting impact with time

In *D. microcarpum*, *K. senegalensis*, *L. lanceolata* and *P. erinaceus*, the impact of wounding completely disappeared within 2 years following bark harvesting: indeed, both area and density of vessel and conductive area returned to their normal pre-wounding value. These observations confirm our hypothesis that at least in these species, wound impact was limited in time and eventually even disappeared. Similarly, *Fagus grandifolia* and *Melia azedarach* formed normal-sized vessels 2 years after wounding (Rademacher et al. 1984; Lev-Yadun and Aloni 1993). Frankenstein and Schmitt (2006) also observed for *Populus tremula* × *P. tremuloides* that modifications in lignin distribution in newly formed xylem elements at wound edges occurred less frequently and finally completely disappeared in a 2-year period following bark harvesting. Even though *M. polyandra* and *M. indica* had a normal conductive area at the end of the study period, their vessel density remained significantly higher and their vessel area significantly lower than in the intact wood that had been formed before wounding. Even if the specific conductive area regains its pre-wounding level, the same does not necessarily apply to hydraulic conductivity (Fig. 8). This is linked to the fact that hydraulic conductivity is proportional to the fourth power of radius and consequently the small vessels do not contribute as much to conductivity as large vessels. In *A. africana*, *B. africana*, *P. kotschyi* and *U. togoensis*, their conductivity system continued to be suppressed during the whole study period. Rademacher et al. (1984) also observed that *Acer saccharum* and *Betula alleghaniensis* still produced much smaller vessels 2 years after debarking. Thus, for these trees, more time is needed to return to normal wood anatomy. Unfortunately, systematic position (same family or genus) of any studied species cannot explain the differences obtained in our results.

Spatial diminishing wound effect on conductive area

In eight of our ten studied trees, the spatial impact of bark harvesting was restricted to a small area around the wound. *L. lanceolata* showed changes in wood anatomy up to 2 cm from the wound's limit, whereas the wood of *K. senegalensis* was even affected on the side of the disc opposite the wound.

This interesting finding suggests that whatever the quantity of bark harvested (20 and 50% of tree circumference), the physiological perturbation of the tree is located closely around the wound, except for *K. senegalensis*. Thus, and thanks to its intact part, the tree continues its growth and physiological development. Except for cases in which a large part of the trunk was wounded (suggesting interruption of water transport), we did not observe severe reduction in the leafy canopy. The latter observation might be explained by recent studies on the three-dimensional vessel network, which gave a better understanding of the pathways of ascent and distribution of water in the plant body (Tyree and Zimmermann 2002; Kitin et al. 2004; Loepfe et al. 2007). They deducted that the xylem was a network of interconnected conduits, with the latter conduits not only connected end to end but also through their side walls (Cruiziat et al. 2002). In this context, having a high connectivity would diminish the negative impact of losing one or several conduits (Loepfe et al. 2007). Our study dealt with the effect of bark harvesting on the anatomy seen on the transversal plane and we observed that the effect of wounding was insignificant 2 cm from the wound. To study the longitudinal effect, Lev-Yadun (2002) decapitated *Pinus pinea* and showed a considerable wound effect up to at least 10 cm below the point of decapitation. He estimated that the distance at which the effects of wounds were not strong enough to change tissue structure ranges from 10 to 40 cm from the point of decapitation. Observations on *Prunus africana* seem to confirm this spatial wound effect: the effect of the wound is spread further in a longitudinal direction than in a transverse direction.

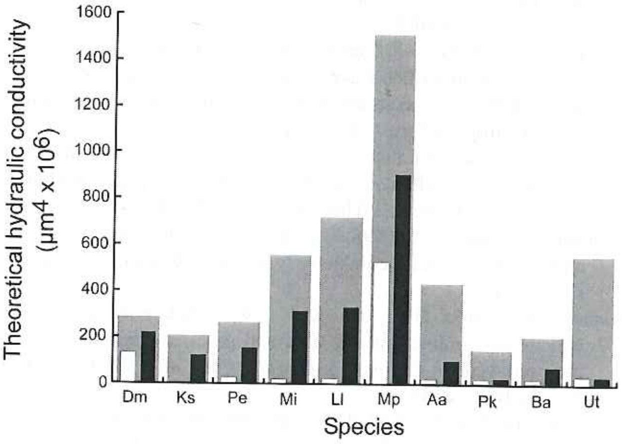

Fig. 8 Simulation of theoretical hydraulic conductivity of xylem based on the Hagen–Poiseuille equation. Vessels were considered as circles and the radius was calculated from the measured area of the vessel. Aa, *Afzelia africana*; Ba, *Burkea africana*; Dm, *Detarium microcarpum*; Ks, *Khaya senegalensis*; Ll, *Lophira lanceolata*; Mi, *Mangifera indica*; Mp, *Maranthes polyandra*; Pe, *Pterocarpus erinaceus*; Pk, *Pseudocedrela kotschyi*; Ut, *Uapaca togoensis*. The *broad gray bar* represents the average conductivity value of the 11 quadrates in the intact wood before bark harvesting. The *white bar* represents the first quadrate produced after wounding. The *black bar* represents the last quadrate produced 2 years after wounding

Relationship between micro and macro levels

A significant positive correlation confirmed the relationship between the specific conductive area and tissue production to close the wound, and delineated two groups of trees (Fig. 6). Group B did not regain a normal-looking conductive area 2 years after bark harvesting, which was explained by vessel size that stayed significantly smaller than before the wound was applied (Fig. 2b). It has been well documented that climate influences vessel formation (size and number) (e.g., Baas et al. 1983; Lindorf 1994; Verheyden et al. 2005). However, in the present study, we may exclude this external factor because all ten species were located in the same area, inside Forêt Classée des Mont Kouffé; hence, they grew under similar climate conditions. After wounding, vessel formation may be mainly considered as controlled by internal factors. As auxin is mainly transported through mature phloem, any damage to the bark results in a greater amount of auxin moving into the cambial region, thus promoting the latter's differentiation into vascular elements (Benayoun et al. 1975). However, Mwange et al. (2003) have shown that auxin content decreases in advanced stages of bark recovery and, as a consequence, lower auxin concentration will induce slower differentiation and therefore initiate fewer and larger vessels (Aloni and Zimmermann 1984; Aloni 1987; Aloni 1992). In view of our results, *A. africana*, *B. africana*, *P. kotschyi* and *U. togoensis*, belonging to group B (weak wound closure), should show altered auxin production even 2 years after bark harvesting. The exact reasons why trees in this group did not regain their normal wood anatomy remain unknown. In this regard, *L. lanceolata* was an exception among the ten species: this tree recovered its conductive area but did not develop new tissues to close the wound. This particular tree was severely attacked by insects suggesting that the tree managed its energy budget by compartmentalizing the wounded stem part to avoid the spread of insect attacks and fungi within the trunk, rather than forming new wood tissue. Infection (fungi, insects, etc.) may further reduce bark recovery rate, which normally is already low in intact trees. A previous study (Delvaux et al. 2009) carried out in the same area showed that *L. lanceolata* is a species with comparatively low bark recovery: only 14.21% of the wound area (mean of 102 individuals) had developed new bark after a 2-year period.

Conclusion

In conclusion, our results indicate that the spatial changes in the wood after bark harvesting were much less important than temporal changes. Recovery of the vessel features toward the condition before wounding is a slow process that requires at least 2 years to complete. Also at 2 cm away from the bark harvesting wound, the specific conductive area was usually not affected. Moreover, in relation to previous studies, we propose that after that kind of bark harvesting, trees may continue with their normal sap flow for photosynthetic activity through their network of interconnected xylem vessels. Trees that showed enough tangential re-growth to close the wound were the same trees, which were able to rapidly produce vessels with similar features (density, size) than before wounding to recover the initial specific conductive area.

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