« Strip-trees »: the life after

Responses to bark harvesting of medicinal tree species from Forêt Classée des Monts Kouffé, Benin

Claire Delvaux

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Dutch translation of the title:
Het leven na "strip-trees".
Reacties op ontschorsing van medicinal boomsoorten uit Forêt Classée des Monts Kouffé, Benin

Front cover: "Mâchonnons les branches en acceptant les mirages de la digestion" N°5
Realisation: Etienne Leclercq

Back cover: The bark regeneration of 12 medicinal tree species two years after debarking
Realisation: Claire Delvaux

Maranthes polyandra	*Afzelia africana*	*Burkea africana*	*Uapaca togoensis*
Lophira lanceolata	*Detarium microcarpum*	*Pterocarpus erinaceus*	*Pseudocedrela kotschyi*
Parkia biglobosa	*Mangifera indica*	*Lannea kerstingii*	*Khaya senegalensis*

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Il faut toujours remercier l'arbre à karité sous lequel on a ramassé de bons fruits pendant la bonne saison.

Ahmadou Kourouma

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La recherche doit avant tout être un jeu et un plaisir.

Pierre Joliot-Curie

To Idriss

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Abbreviations

d.b.h.	diameter at breast height
FAA	Formaldehyde-acetic acid-ethanol
FAO	Food and Agriculture Organization
FCFA	Franc de la Communauté Financière d'Afrique
GLM	Generalized Linear Model
IAA	Indole-3-acetic acid
NTFPs	Non-Timber Forest Products
PEG	PolyEthylene Glycol
SE	Standard Error
WHO	World Health Organization

CHAPTER 1

Outline of the thesis
General Introduction
General objectives

Il y a dans la vie deux sortes de destins. Ceux qui ouvrent les pistes dans la grande brousse de la vie et ceux qui suivent ces pistes ouvertes de la vie. Les premiers affrontent les obstacles, l'inconnu. Ils sont toujours le matin trempés par la rosée parce qu'ils sont les premiers à écarter les herbes qui étaient entremêlées. (…).

Ahmadou Kourouma,
En attendant le vote des bêtes sauvages, Seuil, 1998

Outline of the thesis

Bark is essential to the survival of trees but is also greatly coveted by people. Contrary to an animal, a tree cannot run away from a man armed with a knife and ready to strip it. How to satisfy everyone's needs? This is the challenge behind this work.

For the first time in West Africa the survival of trees after bark harvesting has been investigated in detail, which is especially relevant in the context of sustainable forest management. The **introduction** presents the complex environmental and human context in which the bark management of medicinal tree species must take place. While, until 1970s, the forest was mainly exploited for timber by companies, another resource has progressively gained interest: the non-timber forest products (NTFPs). For the rural populations NTFPs appear to be not only a new source of income but also a fundamental contribution to their subsistence. In this study, medicinal tree species appreciated for the properties of their bark are focused on. The state of knowledge about the ecological response to bark harvesting and about the wound reaction at the anatomical level is exposed.

The **general objectives** of this study are presented.

PART 1 focuses on the ecological responses of 12 medicinal tree species after an experimental bark harvesting carried out on natural populations in the wild. Reactions of trees over a two-year period following bark harvesting are relatively unknown until now, thus these results open the way to future improvement of bark harvesting techniques. The SECOND CHAPTER (published in Journal of Applied Ecology) aims to integrate bark re-growth, vegetative growth and sensitivity to insect attacks in order to elaborate a management strategy for each species through a decisional model. The THIRD CHAPTER (submitted to Biological Conservation) improves the decisional model by adding information on survival, patterns of re-growth and the influence of season, size of the tree and intensity of debarking on re-growth.

The macroscopic phenomenon of bark recovery observed throughout this study is the expression of tissue modification in reaction to injury at the microscopic level. **PART 2** highlights the anatomical features underlying the reaction to bark harvesting. In the FOURTH CHAPTER (submitted to Trees Structure and Function), given the important role of vessels in tree physiology, the temporal and spatial impact of bark harvesting on vessel density, vessel area and total conducting area are investigated. The FIFTH CHAPTER (submitted to Flora) assesses the potential of several anatomical variables to predict the capacity to re-grow after bark harvesting.

The **general conclusion** summarizes what this thesis has allowed us to learn about sustainable management of bark harvesting and change in wood and bark anatomy involved in wound healing. Some perspectives are proposed focusing on 4 species out of 12 that deserve particular attention in future research.

General introduction

"Although malaria was widespread and common, until the early 17th century European physicians had found no truly effective cure, and their patients continued to die. But in the 1630s a possible treatment was found in the forests of the Andes Mountains. In that decade, an Augustinian monk published a notice regarding the treatment: "A tree grows which they call "the fever tree" in the country of Loxa, whose bark, of the color of cinnamon, made into powder amounting to the weight of two small silver coins and given as a beverage, cures the fevers; it has produced miraculous results in Lima," wrote the monk, Antonio de Calancha. (…). The Jesuit Order was the strongest promoter of the bark of Cinchona tree, and it was sometimes called Jesuit's bark or powder. Importation of bark began in the mid 17th century, and continued until the 19th century. The bark was harvested around what is now the Peruvian and Ecuadorian border. (…). Once in Europe, the bark was distributed by a variety of means. Jesuits often gave it away, merchants sold it, and the nobility sometimes used it as gifts. The Spanish presented it to the empress of Hungary, Pope Clement XIV, the Duke of Parma and the general commissioner of holy places in Jerusalem during the period 1772-86. (…). By the mid-19th century the Dutch and English began claiming that the South American supply of cinchona was threatened by the non-sustainable cutting practices of the indigenous harvesters. In 1839, William Dawson Hooker claimed that completely cutting the trees, rather than harvesting pieces of bark, was a better method, because insects would attack cinchona plants that had simply been debarked. On completely cut (or "coppiced") plants, new growth quickly appeared, and could be harvested again in six years. Years later it was also discovered that cut and regrown cinchona had higher levels of the effective alkaloids in its bark, and this method of harvesting became common in many plantations. In fact, the bark of the cinchona tree firstly described by Antonio de Calancha, contains the alkaloid quinine along with several other alkaloids effective against malaria." Adapted from Juliet Burba (1999).

Over a three-century period, most of medicinal tree species continue to be overexploited without a sustainable management.

"Accuse not Nature, she hath done her part;
Do thou but thine, and be not diffident
Of Wisdom, she deserts thee not, if thou
Dismiss her not"   John Milton

FOREST

For centuries, forests have been a source of timber and non-timber products without clear domination for a particular group of stakeholders. It was only after the World War 2 that large-scale industrial timber harvesting and the development of sawmill industries initiated a forestry cycle clearly dominated by timber extraction (Poore 2003). In the 1970s the recognition of the critical role of forests in the life of rural smallholders and local communities refocused attention on multiple values of the forest and stakeholders (Garcia-Fernandez *et al.* 2008). Thus a progressive forestry vision required forest to satisfy multiple stakeholder demands for multiple products (Kant 2004). The implementation of reduced impact logging techniques in the late 1980s was a first practical step to improve timber-harvesting practices by reducing damage to the remaining vegetation (Sist *et al.* 2003). Over time, although forests stay the same, man's perception of forests and how the forest resource is utilized shifts constantly (Wang 2004). Now it is highlighted that the importance of each different user group must be clearly understood before making interventions (FAO 2006a).

NON-TIMBER FOREST PRODUCTS

Non-timber forest products (NTFPs) consist of goods of biological origin other than wood that are derived from forests, other wooded land and trees outside forests. Examples are edible nuts, mushrooms, fruits, herbs, spices and condiments, aromatic plants, fibres, resins, gums, animal products and medicinal plants (FAO 1999). NTFPs have been harvested by human population for subsistence use and trade over thousands of years (Ticktin 2004). The increasing interest in NTFPs started in the 1980s, following social concerns about local communities' needs to increase their share of forest benefits (de Beer & Mc Dermott 1996). NTFPs gained much attention from the conservation world to be sustainably harvested and thus to avoid their overexploitation (Ticktin 2004). NTFPs play a crucial role in meeting the subsistence needs of a large part of the world's population who live in or near forests (FAO 2006a). They also provide small but significant sources of income, particularly for women and for families that do not have access to agricultural markets (Clark 2001). But first of all, NTFPs participate fully in the equilibrium of the whole forest ecosystem (fruits, mushroom, herbs, trees, etc.).

In 1989, Peters *et al.* (1989) first demonstrated that NTFPs not only yield higher net revenues per hectare than timber, but they can also be harvested with considerably less damage to the forest. Moreover, the total revenues generated by the sustainable exploitation of 'minor' forest products (status reflecting their minor importance) are two to three times higher than those resulting from timber exploitation from the forest. Other studies confirmed these first results (e.g. Myers 1988; Godoy & Lubowski 1992; Arnold & Perez 1998; Neumann & Hirsch 2000; Olsen & Larsen 2003; Vodouhe *et al.* 2008). But it is also true that sometimes, the trade of NTFPs results in a low level of income for the poorest section of communities, and thus this commercialization does not tend to alleviate or even reduce the poverty (Neumann & Hirsch 2000; Marshall *et al.* 2006).

Nevertheless, the commercialisation of NTFPs was considered as a "win-win" tool to achieve concurrently conservation and rural development objectives. However, the NTFPs harvesting have to endure a combination of factors which threaten their survival over time:

1. Harvesting from the wild. Most of the NTFPs remain harvested from the wild without control on the exact quantity harvested over time or the place of harvest (e.g.Hamilton 1992; Cunningham 1993; Kuipers 1995; FAO 2005; Olsen 2005; Lange 2006),
2. Overexploitation because of:
   a. growing demand on local, national and international markets (e.g. Cunningham 1993, 1995; Kuipers 1995; Arnold & Perez 2001; Soehartono & Newton 2001; Cunningham *et al.* 2002; Shanley & Luz 2003; Ticktin 2004; Lange 2006),
   b. low income of collectors compared with wholesalers, leading to excessive harvesting of products and therefore overexploit several species (e.g. Neumann & Hirsch 2000; Marshall *et al.* 2006; Vodouhe *et al.* 2008),
3. Unsustainable management. Lack of knowledge of the ecology of tree species providing NTFPs leads to inappropriate management which prevents sustainable harvesting (e.g. Peters 1996; Shanley & Luz 2003; Ticktin 2004),
4. Conflicts of interest for species providing both timber and NTFPs value which can threaten those tree species if timber exploitation does not follow sustainable management (e.g. Shanley *et al.* 2002; Shanley & Luz 2003; Ndoye & Tieguhong 2004).

Some obstacles at the base of the steady but slow increase in the trade (FAO 2006b) may be relieved by giving more attention to some factors essential for a profitable trade. 45 factors (e.g. lack of technical support, financial instrument, infrastructure and equipment for processing, road and transport infrastructure, attractive product presentation, market

valorisation of environmental goods and service) were identified that significantly limit one of the commercialisation processes (production, collection, processing, storage, transport, marketing and sale) (Marshall *et al.* 2003; te Velde *et al.* 2006; Garcia-Fernandez *et al.* 2008). This analysis of the failure throughout the commercialisation process, gives for every level (from local community to government agencies and private sector institution) a general guidance with a great range of solutions useful to improve the success of NTFPs commercialisation.

Over time, the reflection of Peters mimics the situation of the NTFPs harvesting situation today. In 1989, Peters et al. (1989) showed the financial importance of NTFPs and they argued that these products can also be harvested with considerably less damage to the forest than timber exploitation. Few years later, Peters (1996) wrote: "One of the most basic and rarely questioned assumptions underlying much of the current interest in non-timber forest products is that the commercial extraction of NTFPs has little or no ecological impact on a tropical forest". Indeed, by this sentence, Peters recognized that the intensity of subsistence harvesting as traditionally practiced by forest peoples for thousands of years, was usually lower than that of commercial extraction and that the gradual extinction of a plant species over time is rarely a visible phenomenon. Thus following the commercial harvesting which leads to the increasing overexploitation of tree species throughout the world, new concepts arise intended for restoring the natural equilibrium: sustainable harvesting which is the extraction of a forest product in such a way that the harvest has no long-term harmful effect on the reproduction and regeneration of the population being harvested (Hall & Bawa 1993), and multi-use forest management for timber and non-timber forest products which is envisioned as a promising and more balanced alternative to the timber-dominated strategy (Garcia-Fernandez *et al.* 2008).

"Many plants can live hundreds of years without succumbing to diseases or predation. It should come to no surprise that some of the compounds that have enabled plants to survive may also be used to maintain the health and well-being of humans." (Schmidt *et al.* 2008)

A REMARKABLE NTFP: MEDICINAL PLANTS

Plants have been selected and used empirically as drugs for centuries, as traditional preparations in the history of all civilisations. Traditions of plant-collecting and plant-based medications have been handed down from generation to generation (von Maydell 1996) and men people were completely dependent on medicinal herbs for the prevention and treatment of diseases. It was not until the 19th century that man began to isolate the active principles compounds of medicinal plants and one particular landmark was the discovery of quinine from *Cinchona pubescens* bark to treat malaria (Phillipson 2001). Prior to World War 2, a series of natural products isolated from flowering plants became medicine and a number are still used today (quinine from *Cinchona pubescens* bark, morphine from the latex of papaver, digoxin from *Digitalis lanata* leaves, atropine from species of Solanaceae, etc.) (Phillipson 2001). In the post-war years, discoveries of new drugs from flowering plants were made (taxol from *Taxus brevifolia* bark, reserpine from *Rauvolfia serpentina* root as tranquillisers and vinblastine from *Catharanthus roseus* for cancer chemotherapy). Despite these discoveries the impact of phytochemistry on new drug development decreased and the pharmaceutical industry began to concentrate their research on synthetic chemicals. At the end of 1980s, with the renewed interest from Western countries in herbal remedies and the increasingly urgent need to develop new effective drugs, traditionally used medicinal plants have received the attention of the pharmaceutical and scientific communities (Taylor *et al.*

2001). This attention was much more important and urgent in Africa since it is well-known that oral transfer of knowledge is vulnerable to disruption and may result in the loss of valuable ethnomedical information (Fyhrquist 2007). Moreover, documentation of traditional medicinal plants and remedies is becoming increasingly important owing to the rapid loss of the natural habitats and thus the loss of the ingredients of medicinal preparations (Iwu 1993).

Of the 250 000 species of flowering plants, the majority have not been examined in any detailed way for their pharmacological properties (Phillipson 1997). It is difficult to estimate the exact number of plants which are at the origin of medicinal drugs. An analysis of plant derived materials used in prescription drugs found that only 40 species of flowering plants are used as sources of drugs (Farnsworth *et al.* 1986; van Seters 1995). Until 1996, over 45 000 plant samples have been collected by the National Cancer Institute (NCI) contractors, and over 40 000 have been extracted to yield more than 87 000 organic solvent and aqueous extracts (Cragg *et al.* 1996). Over 36 000 extracts have been tested in the anti-AIDS screening, and approximately 10% have exhibited some in vitro activity (Cragg *et al.* 1996). In spite of these progresses, the rising cost of Western medicine means that the people in African countries remain dependent on their traditional medicine. 80% of people in developing countries continue to rely on traditional healers (medicinal practioners) and local medicinal plants for their primary health care (WHO 1995). It is estimated that some 35 000 – 70 000 plant species are used in traditional systems of medicine (Farnsworth *et al.* 1991). Natural products and plant-derived products continue to be excellent sources of new drug candidates, particularly those with traditional medicinal uses that have not been chemically analyzed to date.

Rural communities harvest mostly in the wild medicinal plants sought for their own health care, but this wild harvesting was not detrimental to plant survival as the quantity collected tended to be small and also most of the material collected came from the more common varieties. This is not true for pharmaceutical, phyto-pharmaceutical companies and also the local market which need an increasing amount of specific medicinal plants. It is interesting to note that in the National Cancer Institute, the approval of an agent for clinical development could require 5 to 200 kg of the dried plant material, preferably from the original collection site. Thus to achieve such large re-collections, NCI organizes surveys to determine the abundance and distribution of the plant, as well as the variation in drug content with the season of harvesting (Cragg *et al.* 1996). For example, in the case of the biological tests of taxol, the NCI used no less than 3.25 tons of bark between 1977 and 1987 (Chevassus-au-Louis 2000). This kind of investigation may lead to a risk of over-harvesting and depletion of the wild gene pool. Knowing that many of world's pharmaceutical companies have research programs which investigate plants for their biological activities in the search of new drug entities (Phillipson 1997), we should be aware of the impact of the research of new drugs on forest ecosystems worldwide. Moreover, there is an enormous demand in medicinal plants, for domestic use and for commercial trade, resulting in a huge trade on local, national and international level (Hamilton 1992; Lange 2006). It appears evident that the growing commercial trade of natural products in medicinal plants causes an increasing volume of harvested plants mostly from the wild (Hamilton 1992; Kuipers 1995; Lange 2002).

Given pressure on medicinal plant resources become greater than ever, the sustainable management of medicinal plants harvesting is the primordial issue to avoid overexploitation leading to extinction of species.

Management of medicinal plants: a complex process

The medicinal plant management is complex. On the one hand, target species and harvested amount will depend on the kind of market where it will be sold: i.e. local, national or international markets. On the other hand, the impact of NTFPs harvesting varies depending to

which part (leaves, bark, fruit, root, etc.) of the plant is harvested and may alter biological processes at many levels. The NTFPs harvesting may affect the physiology and vital rates of individuals, change the demographic patterns of plant populations and alter community- and ecosystem-level processes (Ticktin 2004).

The choice of species: for local, national or international trade?

The scale of international trade in medicinal plants is difficult to assess with precision because of the paucity of reliable statistics and trade secrecy (Hamilton 1992). Nevertheless, Lange (2006) investigated the international trade in medicinal and aromatic plants for the period 1991-2003. One of the main features of the international trade of pharmaceutical plants is the dominance of only few countries: about 80% of the worldwide imports and exports are allotted to only 12 countries: USA, Germany, France, Italy, Spain and UK are among the top 12 countries of import and China heads the list of the world's top 12 export countries. In Africa, Cunningham (1996) found that only a limited number of species were traded in significant volumes. This was confirmed a few years later. A study of FAO (2005) showed that in 2002 Africa remained poorly represented on the international export trade in medicinal plants (Table 1.1). African countries exported 44.6 10^3t of medicinal plants (7.6% of global export volume) which is the equivalent of 62.6 million USD (6% of the world export value). As expected, their importation was negligible (Table 1.2) with less than 2% of the global import volume and less than 1% of the global import value.

Table 1.1: World export volume and value of Medicinal plants in 1991 and 2002 (FAO 2005)

Year	Export volume (1000 tons)		Export value (millions US$)	
	1991	2002	1991	2002
World	371.9	583.6	1135.8	1034.8
Developed countries	60.8	132.3	338	407.9
Developing countries	311	451.4	797.8	627
Africa	16.4	44.6	33.7	62.6
Egypt	6	12.7	12.6	11.2
Sudan	2.4	12.7	6.3	16.9
Morocco	4.9	102	11.8	15.3
South Africa	0	5.2	0	12.8
Cameroon	0.9	1.3	0	1.2
Madagascar	0.6	0.5	0.8	0.8

Table 1.2: World import volume and value of Medicinal plants in 1991 and 2002 (FAO 2005)

Year	Import volume (1000 tons)		Import value (millions US$)	
	1991	2002	1991	2002
World	375.1	426.8	1153.5	1128.4
Developed countries	183.8	239.4	543	673.2
Developing countries	191.3	223.5	610.5	455.2
Africa	2	7.7	3.2	7.3
Egypt	1.7	3.9	1.9	2.4
Nigeria	0.2	1.5	0.1	0.8
Morocco	0	0.7	0.1	1.1
Sudan	0	0.7	0	1.3
South Africa	?	?	0.8	1.2

Traded internationally, and harvested from the wild, *Prunus africana* provides the largest collected volume of any African medicinal plant (Hamilton 1992; Cunningham *et al.* 2002). The exportation is exclusively destined to France (Kuipers 1995) but extracts of bark, marketed as Tadenon (France) and Pigenil (Pharmafar, Italy), are used in several European countries to treat early stages of benign prostatic hypertrophy (Hamilton 1992). The rural collectors sell for 70 FCFA per kilo of bark while Plantecam society pays 125 to 280 FCFA per kilo to retailers. It appears clearly that collectors have the lowest income margins while retailers have the highest (Sunderland *et al.* 1999; Cunningham *et al.* 2002). This situation obliges the rural communities to overexploit *Prunus africana* from the wild in order to improve really their livelihood. In the case of *Prunus africana*, the development of domestication is fully justified both in view of the ecological emergency to conserve this species and the stability of trade.

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Unfortunately, an emphasis on international markets often overshadows attention to the local or national trade in many traditionally important products while export markets may not be the most important in terms of contribution to rural income and employment, or of quantities involved (Arnold & Perez 1998; Shackleton *et al.* 2007). Moreover, the destruction of the tropical forest destroys the primary health care network involving plants and traditional healers which replace the hospitals and Western-trained doctors (Balick 1990).

While appreciating that all scales of markets are important, the local market has many advantages. The local demand covers a higher number of species which avoids targeting intensively only few species from the wild and the traditional approach of harvesting is responsible for conserving at least a proportion of the resources (Soehartono & Newton 2001). Moreover, as many markets are growing through both entry of new products and growth in existing trade (Shackleton *et al.* 2007), new sources of income are available for rural communities which allow them to improve the well-being of the whole family. Nevertheless, at each step of the trade chain, no matter how small, the fair price must be established between actors. To compensate for low margins of income, collectors increase the amount of harvested medicinal plants from the wild leading to overexploitation of some species (Vodouhe *et al.* 2008). The importance of a local market supplied by local collectors is highlighted by the fact that exhaustion of a species is rapidly marked. The first indicator for the local collectors is the increase in time to collect the medicinal plant. In fact, local communities did not say that a species has disappeared but that they need to go farther to harvest the plant and thus they need more time. Another indicator is the name of some neighbourhoods. "Agao" came from *Khaya senegalensis* which gave its name to a neighbourhood in Manigri village (Central Bénin) because previously, this species was abundant on this place. Thirty years later, we have to walk 40 minutes before finding the first *Khaya senegalensis*. A similar finding was made by Balick (1990) (Fig 1.1). Thus the added time and effort involved in the collection of medicinal plants is an increased burden for collectors or traditional healers (Balick 1990; Shanley & Luz 2003). If collectors belong to the village, it is possible that they start to cultivate wild plants once that resource has declined to such an extent that transport costs, search time and long distance trade have driven up their price considerably (Schippmann *et al.* 2002). in the San Antonio region of Belize (Balick 1990).

This initiative may be supported and fostered by traditional healers which may play an important role in the conservation around the village. However, in the case collectors and retailers came from abroad, exhaustion of

Fig. 1.1. Time required to reach secondary forest site for medicinal plant collecting. Substantial increase from 1984 to 1988, indicating high level of forest destruction

species in one place may lead them to a geographic shift for their harvesting activities rather to domestication or cultivation (Siebert & Belsky 1985; Peters *et al.* 1987), leaving the rural communities deprived of one of their natural resources useful for their primary health care.

With the aim to conserve the natural resources so essential for the local communities (knowledge of traditional healers, primary health care, new income, etc.) selection of our species was based on the use by local populations and not on the trade for export (See Annexe 1 for the medicinal uses of the 12 medicinal tree species through the literature). Nevertheless, once appropriate harvesting methods have been determined, it would be opportune to share these methods with the industry sectors to assist them in becoming more responsible partners in the medicinal plant trade (Srivastava 2000).

Ecological impact of harvesting?

The exploitation of medicinal tree species has a variable effect depending on the parts harvested. For example, harvesting flowers, fruits and leaves has a significant impact on regeneration and on the population viability (Hall & Bawa 1993; Peters 1994; Witkowski *et al.* 1994; Endress *et al.* 2004; Siebert 2004; Gaoue & Ticktin 2008). However, harvesting bark or roots is more damaging in terms of tree survival (Cunningham 1991; Peters 1994; Witkowski *et al.* 1994; Davenport & Ndangalasi 2002; Geldenhuys 2004; Vermeulen 2006; Geldenhuys *et al.* 2007; Vermeulen 2009). Moreover, most medicinal plants are harvested for more than one reason (Shackleton *et al.* 2002). As suggested for NTFPs (Ticktin 2005), the sustainable management of medicinal trees requires knowledge of how different species respond to different harvesting techniques. Despite the economic importance of bark to local communities e.g. *Khaya senegalensis*, *Mangifera indica*, *Croton cajucara*, *Tabebuia impetiginosa*, *Stryphnodendron barbatiman*, *Trema micrantha*, *Ocotea bullata*, *Waburgia salutaris*, (Peters *et al.* 1987; Shanley & Luz 2003; Botha *et al.* 2004b; Geldenhuys 2004; Assogba 2007) or on the international market e.g. *Prunus africana*, *Pausinystalia johimbe* (Sunderland *et al.* 1999; Cunningham *et al.* 2002) not much information is available on the ecological impacts of bark harvesting (Ticktin 2004), except for a few studies in South Africa (Geldenhuys 2004; Vermeulen 2009) and southern Africa (Geldenhuys *et al.* 2007).

The exploitation of medicinal plants can also affect many levels of forest ecology from individual and population to community and ecosystem. The integration of conservation and use must be present at each level and it is not an option but a necessity (Newton 2008). We have to keep in mind that millions of people depend on exploitation of the forest thus the concept of sustainability means that we should be able to assure the maintenance of viable populations of tree species that are harvested (Newton 2008). The challenge of the scientific and economic community is to find a successful equilibrium between the various needs. That is why we cannot favour one level but have to make a study focusing on all levels of forest ecology (Ticktin 2004). Previous studies approached the trade of NTFPs and the biology of the tree species from a different point of view. Nevertheless, it should not be assumed that all species from a forest considered to be sustainably managed will necessarily themselves be sustainably harvested, in terms of maintaining viable populations (Ticktin 2004; Newton 2008). Consequently, in the case of medicinal plant management it is more essential to pay special attention to the study of the individuals and populations of target species. The review of Ticktin (2004) showed that between 1987 and 2003, more than half of the studies (42/70) that quantitatively assess the ecological implications of harvesting NTFPs was targeted at the population level. Fewer studies concerning various NTFPs (16/70) focused on the individual tree level. In the case of bark harvesting surveys, very few studies were undertaken either at population level or individual level. Gaoue and Ticktin (2008) estimated that the debarking made by rural communities on *Khaya senegalensis* growing within various kind of forest (gallery forest, dense dry forest, woodland) in Benin had no significant effects on reproductive performance of these populations. Guedje *et al.* (2007) investigating the effect of traditional tree debarking on *Garcinia lucida* population dynamics, showed that the actual exploitation of *G. lucida* bark does not jeopardize the existence of the entire population

because the mean population growth rate obtained was above 1. Despite the high interest for *Prunus africana* many years ago, to the best of my knowledge, any experimental bark harvesting study was made at individual level until the studies of Geldenhuys (2004), Geldenhuys *et al.* (2007) and Vermeulen (2009).

"The potential for strengthening conservation efforts ranges from low to high, depending on whether the extraction of the resources can be sustainably managed over the long term or is simply exploited for short-term benefits by collectors and an industry that has little interest in ensuring a reliable supply in the future. Conservation potential is minimal if the end products are derived from synthetic processes or from plantations developed outside the original area of collection." (Balick 1994)

To date, little information is available on the mechanisms underlying the observed effects of harvest (Ticktin 2004).

TREE TRUNK = BARK + CAMBIUM + WOOD

The vascular cambium is a meristematic tissue that encircles the stem of all woody plant species. The cambium not only maintains itself, but also generates radial files of secondary xylem cells to its inside, and radial files of secondary phloem cells to its outside (Fig. 1.2) by repeated cell division (Fig. 1.3) (Chaffey 2002).
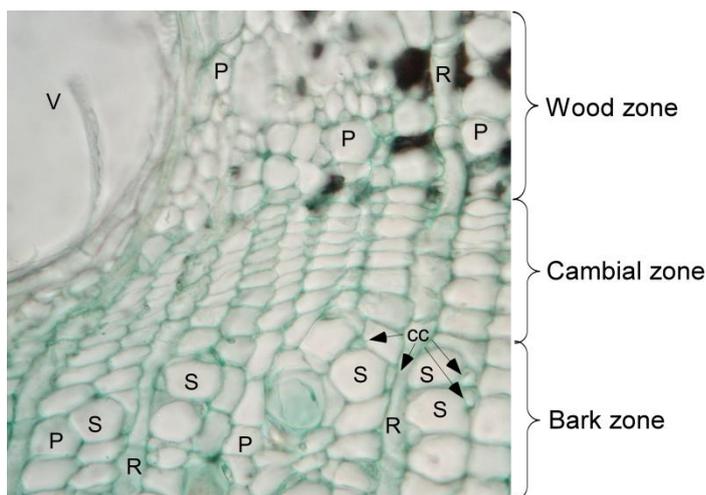
Fig. 1.2 Transverse section of vascular tissues and cambial zone. Theoretically, the cambium is a single layer of cells, called initial cells; practically, it is difficult to distinguish the initials from their still-undifferentiated daughter cells (of secondary xylem and phloem cells) thus several cell layers are called cambial zone. Other details: V, vessel, P, parenchyma cell, R, ray, S, sieve element, cc, companion cell.
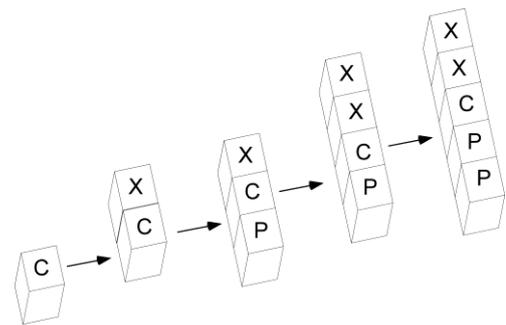
Fig 1.3 Schematic diagram illustrating the division of cambium cell (C) to produce xylem cells (X) by centripetal differentiation and phloem cells (P) by centrifugal differentiation.

The phloem together with the periderm forms the bark tissue which is of primary importance because its shields the xylem from the environment, mechanical injuries and infectious microorganisms (Biggs 1992). The non-technical term bark includes all tissues located outside the vascular cambium (See Annexe 2 for the macroscopic description of the 12

species focused on this study). The periderm is a protective layer of secondary origin and is constituted by three tissues. The phellogen (or cork cambium) is the meristematic tissue that produces phellem (or cork) to the outside and phelloderm (or cork parenchyma) to the inside of the stem. The phellem cells generally have a layering of suberin in their walls. Suberin contains a waxy substance, which forms a barrier to microbial penetration and makes the cells mostly impervious to water and unable to exchange gasses and nutrients. Hence these cells soon die. The phelloderm is a tissue resembling phloem parenchyma where all cells can remain alive because they have unthickened and unspecialized cell walls and, hence, can exchange gases and obtain nutrients. Within the phloem (Fig.1.4), the principal components of the axial system are sieve elements (sieve-tube elements with their companion cells), fibres, and parenchyma cells. Ray parenchyma is the only tissue of the radial system. Following Evert (2006), the phloem can be differentiated into both the conducting phloem and the non-conducting phloem. The conducting phloem is involved with long-distance transport from the moment the sieve element is enucleate and sieve-area pores are open. The non-conducting phloem is the older part of the phloem where sieve area pores are clogged and the sieve elements have ceased to function after one or several seasons. These sieve elements have sieve area either covered with a mass of callose or entirely free of this substance and collapsed. This part contains both dead sieve elements and the living axial and ray parenchyma cells storing starch, tannins, and other substances. The companion cells and some parenchyma cells die too when their associated sieve elements die.
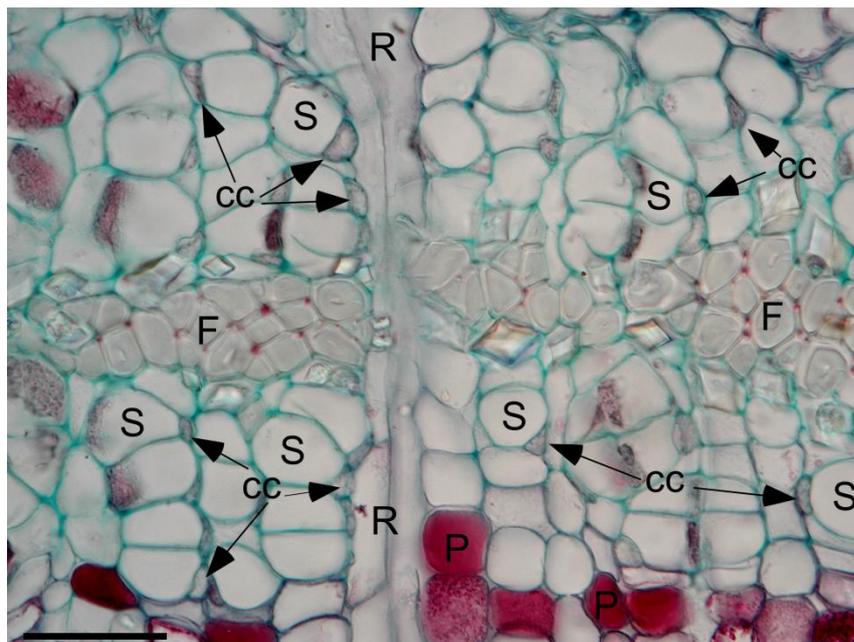
Fig. 1.4 Transverse section of secondary phloem (*Lannea kerstingii*) showing the axial system: sieve element (S), companion cell (cc), parenchyma cell (P) and fibers (F); and the radial system: rays (R). Scale bar: 50μm

Because of the relatively narrow width of the yearly increment of phloem and its usually short functioning life (from one to several seasons), the layer of conducting phloem occupies only a small proportion of the bark (Roth 1981; Evert 2006). The conducting phloem width is not uniform between species as was for instance shown across 259 species studied in Venezuela Guayana (Roth 1981): the thickness of the conducting phloem ranged from 0.2 to 4 mm with a peak at 0.5 mm (15% of studied species), at 1 mm (30% of studied species) and at 2 mm (21% of studied species). Of this conducting phloem 25% to 50% is occupied by sieve tubes

in woody angiosperm (Evert 2006). Unfortunately, the bark structure is still handled as a "stepchild" of tree anatomy, especially when tropical trees are concerned (Roth 1981). Chemical properties, such as tannin and alkaloid content, are studied without knowledge of the bark anatomy. Thus bark becomes as important as that of the wood that it is necessary to increase the number of studies on this topic.

The secondary xylem, called wood, is also composed of two systems (Fig. 1.5). The axial system contains vessels, fibres and parenchyma cells, and the radial system consists of living parenchyma cells (called ray cells). Vessel elements are more or less elongated cells that have lignified secondary walls and are nonliving at maturity. Vessel elements have perforation plates, which are areas lacking both primary and secondary walls. Perforations generally occur at the end walls, joining the vessel elements end-to-end, forming long, continuous tubes called vessels. In angiosperms, vessels together with sieve tubes (within the conducting phloem), form a continuous vascular system extending throughout the plant body (Evert 2006). The sieve tubes are the principal food-conducting tissue, and the vessels are the principal water-conducting tissue (Mohr & Schopfer 1995).
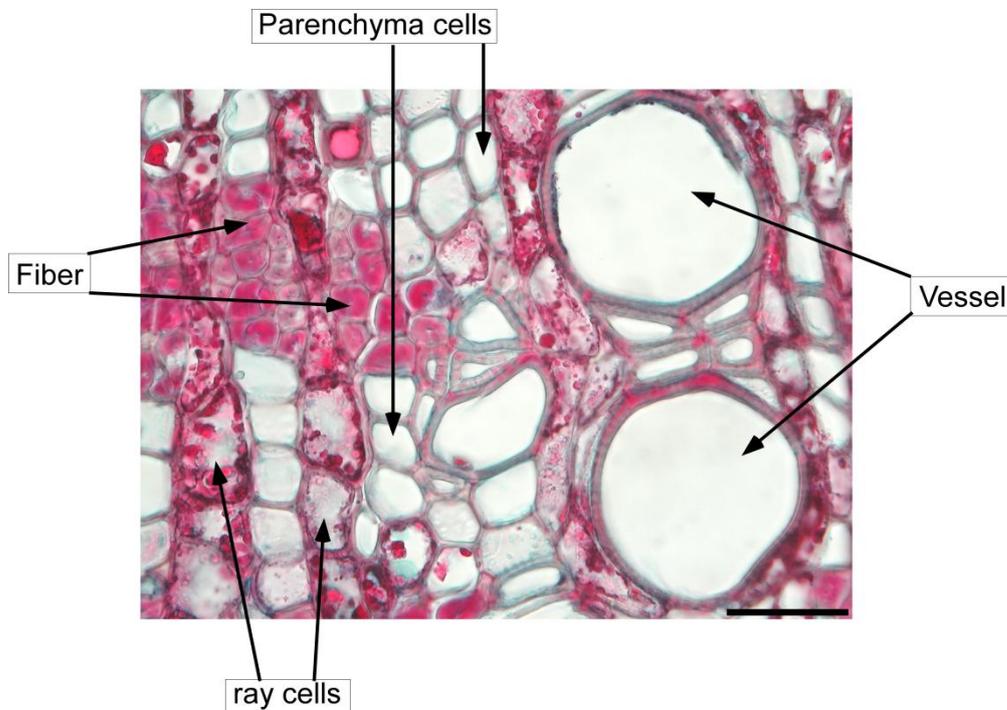
Fig. 1.5 Transverse section of secondary xylem (*Lophira lanceolata*) showing the axial system: vessel, fiber and parenchyma cells and the radial system: ray cells. Scale bar: 50μm

Wood formation in trees growing in temperate or tropical climatic regions, depends on the combined action of intrinsic and extrinsic factors (e.g. Fritts 1976; Sass & Eckstein 1995; Borchert 1999; Schmitt *et al.* 2000). Indeed, the continuous development of new vascular tissues enables regeneration of the plant and its adaptation to changes in environment (Aloni 2004). The control of vessel diameter is an important parameter for assessing the ascent of water and minerals from roots to leaves and the adaptation of plants to their environment (Aloni 1987). There is evidence that the differentiation of vessels is induced by one major hormonal signal, namely, the auxin indole-3-acetic acid (IAA), produced mainly by young leaves (Aloni & Zimmermann 1983). High auxin concentrations induce narrow vessels because of their rapid differentiation, allowing only limited time for cell growth. On the

contrary, low IAA concentrations result in slow differentiation, which permits a longer cell expansion phase before secondary wall deposition, and thereby results in wider vessels (Aloni 2004). Both the diameter and the density of vessels directly influence conductivity (Lovisolo & Schubert 1998; Reyes-Santamaria *et al.* 2002; Christensen-Dalsgaard *et al.* 2007; Sellin *et al.* 2008). Thus, wide vessels are much more efficient water conductors than narrow vessels (Zimmermann 1983). As trees consume large amounts of water, they have to develop mechanisms for protection against disturbance of their water balance. Increased vessel diameters increases efficiency of water conduction dramatically (owing to decrease in hydraulic resistance), but at the same time it decreases hydraulic safety (Zimmermann 1983). For example, climate (i.e. drought, heavy rainfall, flooding) may influence size and density of vessels (e.g. Baas *et al.* 1983; Lindorf 1994; Yanez-Espinosa *et al.* 2001; Preston *et al.* 2006). Smaller vessels contribute to a safer water-conducting system and are an adaptive mechanism to protect trees against drought stress (Aloni & Zimmermann 1984; Verheyden *et al.* 2005). Injury damaging the vessel wall may also disturb the conductivity because air is drawn into the injured vessels, blocking them permanently (Zimmermann 1983). Consequently, wounding induces regenerative vessels that are short and narrow. They are therefore safer than normal vessels and have an adaptive value in case of repeated injury (Aloni & Zimmermann 1984).

RESPONSE TO TREE TRUNK WOUNDING

Whether bark harvesting concerns only a piece of bark or girdling (100% of circumference debarked trunk), wounds damage the food- (phloem) and water-(xylem) conducting tissues. Wounds also expose the inside of the tree to micro-organisms, primarily bacteria and fungi that may infect and cause discoloration and decay of the wood. Given rapid healing of wounds is vitally important for plants (Mohr & Schopfer 1995; Eyles *et al.* 2003), research on wound responses of trees is required in order to understand the processes that favor or impede the recovery of the bark. After bark harvesting, trees react in two ways to heal the wound: a protection reaction and a wound closure reaction.

Protection reactions

Large wounds may never completely close, but they may heal from the inside (Garret & Shigo 1978). Trees respond to wounding by "compartmentalizing" the wounded area to limit the spread of microorganisms which is described in the so-called CODIT model (Compartmentalization Of Decay In Trees) (Shigo 1984b). The formation of a reaction zone composed of modified cells with increased resistance against the inflow of air, desiccation and attack by microorganisms provides a boundary between affected and unaffected tissue (Schmitt & Liese 1993). Chemical barriers keep out most wood-destroying microorganisms and the nature of chemical products varies from species to species. When some tree species are injured the parenchyma surrounding the vessels balloons out its contents into the vessels and a tylosis is formed (Schmitt & Liese 1993; Evert 2006). The tylosis, belonging to the parenchymatic system, blocks the vessel to limit spread of microorganisms and air through the vascular system (Clerivet *et al.* 2000; Sun *et al.* 2006). Amorphous and fibrillar material produced by parenchyma cells may also be observed in vessels and fibres in the reaction zone (Schmitt & Liese 1990, 1993). Suberization improves the resistance to fluid diffusion that contributes to the effectiveness of compartmentalization after wounding (Biggs 1985, 1987; Schmitt & Liese 1993; Hawkins & Boudet 1996). Suberin is deposited as a typical lamellar layer both in axial and radial parenchyma cells and in tyloses, thus suberized cells form a continuous zone internal to the necrotic tissue near the wound surface (Pearce & Holloway 1984; Schmitt & Liese 1993).

Not only do the trees make the existing wood surrounding the wound unsuitable for spread of decay organisms, inflow of air and desiccation; they also try to close the injury from the outside and then continue generating new tissues.

Closure reactions

When bark is harvested so that underlying wood is completely exposed to air, wound closure can begin either from the margins of the wound (edge growth) or/and from the entire exposed surface of the wounded xylem (sheet growth). Moreover a variety of living tissue is involved in the process of wound closure: phloem parenchyma, xylem parenchyma, immature xylem and cambial zone. To date, the wound closure mechanism was only studied in a few species and all of them came from the temperate zone (e.g. Li *et al.* 1982; Rademacher *et al.* 1984; Li & Cui 1988; Biggs 1992; Lev-Yadun & Aloni 1993; Novitskaya 1998; Oven & Torelli 1999; Oven *et al.* 1999; Grünwald *et al.* 2002; Stobbe *et al.* 2002; Frankenstein *et al.* 2005). Nevertheless whatever the location (edge growth or sheet growth) and tissue (phloem or xylem parenchyma, meristematic tissue, immature xylem), thanks to these excellent previous studies, we can describe a potential mechanism including four stages: (i) formation of uniform parenchymatic callus tissue by divisions of undifferentiated xylem, phloem as well as cambial cells according to species; (ii) formation of the continuous ligno-suberised layer on the entire surface of the callus which is necessary and essential for stage 3 ; (iii) formation of a wound cambium within the parenchymatous zone, (iv) production of new xylem and phloem cells. The amount and rate of tissue production following wounding varies both between and within tree species (Gallagher & Sydnor 1983; Martin & Sydnor 1987; Neely 1988; McDougall & Blanchette 1996; Geldenhuys 2004; Vermeulen 2006; Geldenhuys *et al.* 2007; Vermeulen 2009). The source of callus tissue also varies to some extent. Frankenstein *et al.* (2005) specified that with *Populus tremula* x *Populus tremuloides*, cells of callus mostly dedifferentiated from phloem parenchyma cells (Frankenstein *et al.* 2005). In other tree species like *Fagus sylvatica* and *Quercus robur*, callus formation started mainly from living cells of the original vascular cambium (Grünwald *et al.* 2002). In *Abies alba*, *Picea abies*, *Pinus sylvestris* and *Larix decidua*, callus was formed from both cambial cells and phloem parenchyma (Oven & Torelli 1999).

"Imagination is more important than knowledge. For knowledge is limited to all we now know and understand, while imagination embraces the entire world, and all there ever will be to know and understand."

Albert Einstein

General objectives

To address the issues of bark harvesting impact, we conducted a case study of 12 medicinal tree species in Benin. This research uses an interdisciplinary approach, combining ecological and anatomical methods to understand how tree species respond following bark harvesting and what anatomical variable(s) favour the bark regeneration.

The ecological objective. We assessed specific responses to debarking by different medicinal tree species and tested how different techniques of bark harvesting affect each species, notably in terms of bark re-growth location (from the edge of the wound or/and from the wound surface area), survival and pattern of re-growth over time. We also assessed the inter-specific differences: the various species presented a wide range of responses to debarking which allowed us to propose a decision tree model helpful for forest managers.

The anatomical objective. We assessed the changes of vessel features before and after wounding. Various features differed among tree species, so that the study of vessel appears to be a good criterion for evaluating the tree ability to produce tissue in order to close the wound. We also assessed at least one anatomical variable (within wood, cambial or phloem zone) which plays an essential role in the process of wound closure. This variable could be a helpful indicator of the regeneration potential in other species.

Someone's sitting in the shade today because someone planted a tree a long time ago.

Warren Buffet

PART 1

KEYS FOR THE SUSTAINABLE MANAGEMENT OF BARK HARVESTING

CHAPTER 2: RECOVERY FROM BARK HARVESTING OF 12 MEDICINAL TREE SPECIES IN BENIN, WEST-AFRICA

CHAPTER 3: INFLUENCE OF SEASON, STEM DIAMETER AND INTENSITY OF DEBARKING ON SURVIVAL AND BARK RECOVERY RATE OF 12 MEDICINAL TREE SPECIES, BENIN

CHAPTER 2

Recovery from bark harvesting of 12 medicinal tree species in Benin, West Africa

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Summary

1. Introduction. The growing interest in medicinal plants from both international industry and local markets requires management of tree bark harvesting from natural forests in order to prevent inappropriate exploitation of target species. This study was designed to determine the bark re-growth response of a selected number of medicinal tree species as a basis for the development of an optimal bark harvesting method.

2. Methods. In 2004, bark was harvested from 925 trees belonging to 12 species in 38 sites in a dry forest in Benin, West Africa. Two years later, the response of trees to bark harvesting was examined with respect to re-growth (edge or sheet), development of vegetative growth around the wound, and the sensitivity of the wound to insect attack.

3. Main results. Two species, *Khaya senegalensis* and *Lannea kerstingii,* showed complete wound recovery by edge growth. At the other extreme, *Afzelia africana*, *Burkea africana* and *Maranthes polyandra* had very poor edge growth. *Maranthes polyandra* showed good sheet growth, whereas the other 11 species had none or poor sheet growth after total bark harvesting. In contrast, partial bark removal allowed better sheet growth in all 12 species studied. Insect sensitivity was species-specific. Insect attacks were negatively correlated with non-recovered wound area, but there was a marked species effect for the same rate of regeneration. *Lannea kerstingii* and *Khaya senegalensis* had very good and similar re-growth, but *L. kerstingii* was very susceptible to insect attack, whereas *K. senegalensis* appeared to be very resistant. Only a few individuals developed vegetative growth, and each tree usually developed only one or two agony shoots, but there was no significant difference between species.

4. Conclusion. This is the first study to provide data on the ability of trees to close wounds after bark harvesting in West Africa. We report large variability in the response of different species to our bark harvesting technique, and identify just 2 out of the 12 study species as suitable for sustainable bark harvesting. Based on our results, we developed a decisional step method to help forest managers select the best techniques for managing medicinal tree species as an alternative to bark harvesting, e.g. coppice management, harvesting leaves instead of bark, stand establishment, and collaboration with timber companies.

Key-words: bark, forest management, insect attack, medicinal trees, re-growth, sustainable harvesting, vegetative growth, West Africa, wound.

Introduction

Two of the many threats to medicinal plant species are the loss of local knowledge about their use and the loss of species from the wild due to overharvesting. The sum of human knowledge about the types, distribution, ecology and management of medicinal plants, and methods for extracting the active components shows rapid decline (Hamilton 2004). This loss of local knowledge has been ongoing for hundreds of years. In recent decades, many ethnobotanical and ethnopharmaceutical studies have been undertaken to document and describe traditional herbal products and to validate their use (Light *et al.* 2005). During that period, however, many plants have become threatened due to a lack of local control on harvesting levels. The global demand for herbal medicine is large, and steadily growing (Srivastava 2000; Light *et al.* 2005), which has caused some valued indigenous plant species to become threatened or, in some cases, to go extinct (Williams *et al.* 2000; Shingu 2005). Medicinal plants can have other uses, e.g. timber, firewood, fodder, etc., and the threat of over-harvesting may be due, at least partly, to gathering for other purposes (Hamilton 2004).

There is an urgent need to develop appropriate conservation strategies to promote sustainable use of medicinal plants through improved harvesting techniques, cultivation and monitoring (Cunningham 1991; Schippmann *et al.* 2002; Botha *et al.* 2004b; Belcher *et al.* 2005; Light *et al.* 2005; Geldenhuys *et al.* 2007).

The sustainable management of medicinal tree species is far from simple. First, the exploitation of these species has a variable effect on the plants themselves, depending on the parts harvested. For example, harvesting flowers and fruits has a significant impact on regeneration and on the population viability (Hall & Bawa 1993; Peters 1994; Witkowski *et al.* 1994; Endress *et al.* 2004; Gaoue & Ticktin 2008). However, harvesting bark or roots is more damaging in terms of tree survival (Cunningham 1991; Peters 1994; Witkowski *et al.* 1994; Davenport & Ndangalasi 2002; Geldenhuys 2004; Vermeulen 2006). Secondly, most medicinal plants are harvested for more than one reason (Shackleton *et al.* 2002). As suggested for non-timber forest products (Ticktin 2005), the sustainable management of medicinal trees requires knowledge of how different species respond to different harvesting techniques. In general, the production rate of the resource will determine how much of it can be used sustainably (Geldenhuys 2004).

This study examined the impact of bark harvesting on trees in order to promote sustainable management of medicinal trees. The term bark is generally considered to include all tissues outside the vascular cambium, regardless of their composition (Junikka 1994). The complexity of the bark tissues derives from the presence of a mixture of dead and live tissues. The rhytidome is the dead outer part of the bark that serves as a physical barrier and protects the tree against attack by herbivores, insects, fire, and fungi. The live tissue called phloem constitutes the inner bark, which is also called non-collapsed secondary phloem because it is the part of the secondary phloem that contains open and non-collapsed sieve elements (Trockenbrodt 1990). Elaborated sap is transported from leaves to roots through these non-collapsed sieve elements. A simple wound in the bark can easily disrupt the physiological functioning of a tree; continuous development of new vascular tissues (Aloni 1987) allows the regeneration of the wounded part of the tree.

Bark from numerous species has long been used by humans for the treatment of diseases, such as fungal skin infections (*Milicia excelsa*), fever (*Alstonia constricta*), malaria (*Cinchona officinalis*) and benign prostatic hyperplasia (*Prunus africana*). Sustainable bark harvesting techniques vary among species, depending on the ability of individual trees to survive harvesting and recover from the inflicted wound. Throughout Africa, only a few studies have assessed the ability of trees to regenerate bark following harvesting. In Cameroon, Cunningham & Mbenkum (1993) showed that *P. africana* achieves complete bark re-growth

of the bark after ring-barking. Similar studies in Nigeria (Fasola & Egunyomi 2005) indicate that *Alstonia boonei*, *Entandophragma angolense*, *Khaya grandifolia*, *Khaya senegalensis* and *Spondias mombin* belong to the fast re-growth group, whereas the bark of *Adansonia digitata*, *Gliricidia sepium*, *Newbouldia laevis* and *Theobroma cacao* have relatively slow re-growth. In South Africa, *P. africana*, *Ocotea bullata* and *Warburgia salutaris* show good re-growth; in contrast, the bark of *Rapanea melanophloeos* shows no re-growth (Vermeulen 2006; Geldenhuys *et al.* 2007). These results show clearly that the ability to regenerate bark after harvesting is species-specific.

In Benin, a nationwide ethnobotanical survey (Adjanohoun *et al.* 1989) showed that bark represents 10.5% of medicinal plant products, and that 31.5% of tree species occurring in the country are used for their bark. The present study was done in Benin and assessed the impact of bark harvesting on 12 tree species used by local communities living around the Forêt Classée des Monts Kouffé, central Benin. We hypothesized that these 12 medicinal tree species (Table 2.1) might differ in their ability to recover from wounding. More specifically, the objectives of the study were: (i) to compare the regenerative ability (edge and sheet re-growth) for the 12 species; (ii) to assess the impact of bark harvesting on vegetative growth (shoot development around the wound) and insect attack; and (iii) to develop a species-specific method for sustainable management of bark harvesting.

Materials and methods

STUDY AREA

The study was conducted in the Forêt Classée des Monts Kouffé (8°30' - 8°52' N, 1°40' - 2°27' E) in central Benin, West Africa (Fig.2.1). This area covers 180,300 ha within the Sudano-Guinean phytogeographic region. The average monthly temperature is 21.0°–33.2°C and the average annual precipitation is 1190.7 mm. The study was carried out in woodland.

STUDY SPECIES

As a first step, a large number of medicinal tree species were selected in order to be able to compare a sufficient diversity of response to bark harvesting. Interviews were held with traditional healers and local populations in order to learn their preference for tree species used for health care (Bockx 2004), and 12 of the most frequently used species of trees were chosen for the study (Table 2.1).
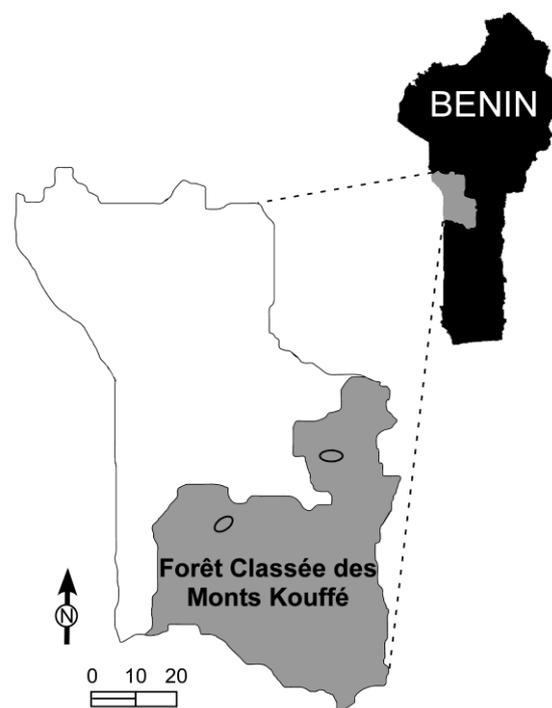
Fig. 2.1: A map of the study area in the Forêt Classée des Monts Kouffé in Department Donga (Benin). Circles represent the distribution of the two field stations where the 38 sites were chosen.

Table 2.1: The 12 tree species in this study. The number of individual trees (N) and the range of diameter at breast height (d.b.h.) values are given.

Species	Family	N	Height (theoretical) (m)	d.b.h. (theoretical) (cm)	d.b.h. (measured) (cm)
Afzelia africana Sm.	Fabaceae (C)	68	25-30	40-60 (>100)	15.6-41.7
Burkea africana Hook.	Fabaceae (C)	78	10-12 (20)	40-60 (>80)	11.6-44.0
Detarium microcarpum Guill. & Perr.	Fabaceae (C)	82	08-10	20-30 (50)	13.5-45
Khaya senegalensis (Desv.) A. Juss.	Meliaceae	73	25-35	40-70 (130)	12-36.4
Lannea kerstingii Engl. & K. Krause	Anacardiaceae	48	12	40-60 (70)	17-44.9
Lophira lanceolata Van Tiegh. ex Keay	Ochnaceae	102	08-10	20-30 (40)	14.9-36.4
Mangifera indica L.	Anacardiaceae	86	10-15 (30)	20-30 (60)	12.2-47.2
Maranthes polyandra (Benth.) Prance	Chrysobalanaceae	53	06-08	15-25 (40)	12.8-35.1
Parkia biglobosa (Jacq.) R. Br. ex G. Don	Fabaceae (M)	44	10-15	30-50 (150)	14-49.5
Pseudocedrela kotschyi (Schweinf.) Harms	Meliaceae	93	09-12	20-30 (40)	13.3-40.4
Pterocarpus erinaceus Poir.	Fabaceae (P)	96	08-12	30-50 (100)	13.5-40.5
Uapaca togoensis Pax	Euphorbiaceae	102	10-15	20-30 (50)	12.3-48.2

(C): Caesalpinioideae; (M): Mimosoideae; (P): Papilionoideae

SAMPLING DESIGN AND HARVESTING TREATMENT

Sites with sufficient numbers of the chosen species were selected, and all were situated within the forest and away from agricultural activity. Only healthy trees (no previous bark harvest) were selected. Bark was harvested from a total of 925 trees from 38 sites in the dry season (February and March) and in the rainy season (September and October) in 2004. The number of individual trees per species is given in Table 2.1. The reasons for differences in the number of trees per species were: (i) difficulty in finding trees with an appropriate diameter according to the species morphology. In the wild, it is rare to find examples of *B. africana*, *D. microcarpum* or *M. polyandra* with a diameter at breast height (d.b.h.) > 30 cm. (ii) Some species (*A. africana, K. senegalensis, P. kotschyi,* and *P. erinaceus*) had been heavily harvested for timber and so trees with d.b.h. > 30 cm were scarce in the study area. (iii) Some species naturally have a sparse distribution (e.g. *L. kerstingii,* and *P. biglobosa*) and finding a sufficient number of these species would have required excessive travelling and time.

Bark was collected from all species according to the same protocol. Wounds were usually made at 1 m stem height. The wound consisted of a rectangular piece of bark 60 cm vertically, and the lateral extent of the wound varied between 5 cm and 61.8 cm, depending on the diameter of the tree, affecting from 20% to 100% (girdling or ring-barking) of its circumference. Two treatments were used on each tree in order to compare different bark harvesting techniques and their impact on the ability of a tree species to recover from the wound. In one treatment the bark was harvested only thinly in the upper 30 cm half of the wound to determine if incomplete debarking favours wound closure. Bark was peeled from the trunk in such a way that a thin layer of inner bark and the cambium were not removed. The amount of bark left on the trunk was much the same for all trees thus treated, and the surface area harvested was 20 – 50% of the total wound area. To guarantee uniform treatment, all samples were collected by the same three people. In the second treatment, no bark or cambium was left on the lower 30 cm of the wound so that the wood was completely exposed to air. This is the method used by commercial bark harvesters. For total bark removal, the wound was inflicted with a cutlass machete cutting the bark down to the cambium level and then removing it from the trunk by tapping with a hammer.

MEASUREMENTS

Two years after bark harvesting, five measurements were taken for each wound. Individual tree response was then classified according to the score levels given in Table 2.2.
1. Sheet growth, i.e. live tissue re-growth on the surface of the wound. (i) On the lower 30 cm of the bark treatment, tracing paper was used to copy the surface area of sheet growth on the wound. (ii) On the upper 30 cm of the wound, the sheet growth percentage was estimated visually as pieces of bark remaining prevented the use of tracing paper. The results were expressed as a percentage of re-growth area.
2. Edge growth, i.e. the surface of live tissue developing from the edge of the wound. This measurement was made only on the lower 30 cm of the wound; three horizontal measurements were made from fixed points on both sides (left and right) of the wound to obtain the mean edge growth value. The results were expressed as a percentage of re-growth area.
3. Insect holes in the lower 30 cm of the wound were counted.
4. The number of agony shoots around the wound was counted. An agony shoot was defined by Geldenhuys *et al.* (2002) as a vegetative shoot developing around a wound in response to wounding.

Table 2.2: Description of repartition into four score levels of four variables describing resistance and response of trees after bark harvesting: edge growth and sheet growth (percentage of recovered area), resistance to insect attack (number of holes/tree) and production of agony shoots (percentage trees with agony shoots).

Levels	Edge growth (%)	Sheet growth (%)	Resistance to insect (N)	Agony shoot (%)
4 = very good	> 50	> 50	0	> 50
3 = medium	11-50	11-50	1-20	21-50
2 = poor	1-10	1-10	21-50	1-20
1 = nul	0	0	>50	0

DATA ANALYSES

To compare sheet growth and edge growth between species, individual scores were calculated according to the levels given in Table 2.2. Ordinal score levels were compared between species by a proportional-odds logit model using the polr procedure in R environment (2005). A general linear model (GLM) with a quasi-Poisson error distribution was used to determine the effects of species and re-growth ability on the number of insect holes. The surface of the non-regenerated area was used as a proxy of the tree regeneration rate, and was calculated as the sum of the sheet and edge re-growth surfaces. The effects of regeneration rate and species were added sequentially for adjusting species effects for different regeneration rates. Also, a GLM with a quasi-Poisson error distribution was used to determine the effect of species on the number of agony shoots.

Results

SHEET GROWTH

Comparison of sheet growth after complete bark removal clearly showed the re-growth to be poor to non-existent for all of the 12 species (Fig. 2.2). *M. polyandra* and *P. erinaceus* had slightly better sheet growth compared to the other ten species (proportional-odds logit model on score levels, $P < 0.05$) but both species had a high intra-specific variability (Fig. 2.2). *M. polyandra* had the best sheet growth: 58.2% of trees showed a re-growth process but the surface of the re-growth area varied from 1.3% to 98.7%. There was no sheet growth for *B. africana*, *M. indica* or *P. kotschyi*, and ~90% of the specimens of the other seven species had no sheet growth. Complete wound closure by sheet growth was not observed after complete bark removal.

In contrast, sheet growth after partial bark harvesting was more successful in completing wound closure (Fig. 2.2). In *K. senegalensis* and *L. kerstingii*, 74.1% and 55.3% of trees were able to close the wound completely, whereas only 14.8% and 31.6% of these trees, respectively, had no sheet growth. On the other hand, more than 55% of individuals of seven species showed no sheet growth. Among these species, *L. lanceolata*, *P. biglobosa* and *U. togoensis* had a high intra-specific variability (Fig. 2.2), and some trees had a sheet growth of > 75% of the wound area. Good sheet growth after partial bark harvesting was observed also in *P. erinaceus*, although few trees had achieved complete wound closure after two years.

There was significantly more sheet growth after partial bark removal than there was after total bark harvesting for all 12 species (proportional-odds logit model on score levels, $P < 0.005$; Fig. 2.2). *K. senegalensis*, *L. kerstingii*, *P. erinaceus*, and *M. indica* had the best recovery rates, with a mean sheet growth of 83.3%, 65.5%, 49.3%, and 39.2% of the wound area, respectively, after a partial harvesting versus a mean sheet growth of 1.9%, 1.2%, 7.7%, and 0%, respectively, after total bark harvesting. It is interesting to note that *B. africana*, *P. kotschyi* and *M. indica*, which had no sheet growth after total bark harvesting, had significant sheet growth after partial bark removal, although it was poor for *B. africana* and *P. kotschyi* (3.14% and 11.12% of total wound area, respectively).

EDGE GROWTH

Edge growth was variable among species (Fig. 2.3). *K. senegalensis* and *L. kerstingii* presented a significantly opposed reaction compared with *M. polyandra*, *A. africana* and *B. africana*. Indeed, *K. senegalensis* and *L. kerstingii* had the highest mean edge recovery rate of 88.8% and 80.4%, respectively. After two years, only these two species closed their wounds completely through edge growth: 48.9% of all *K. senegalensis* and 35.3% of all *L. kerstingii* trees showed full recovery (Fig. 2.3). *M. polyandra*, *A. africana* and *B. africana* had the poorest edge growth, and > 60% of these species had no edge growth. *P. erinaceus, P. kotschyi*, *P. biglobosa* and *M. indica* had a mean edge recovery rate of 23.1 – 40.1% and high intra-specific variability (Fig. 2.3).
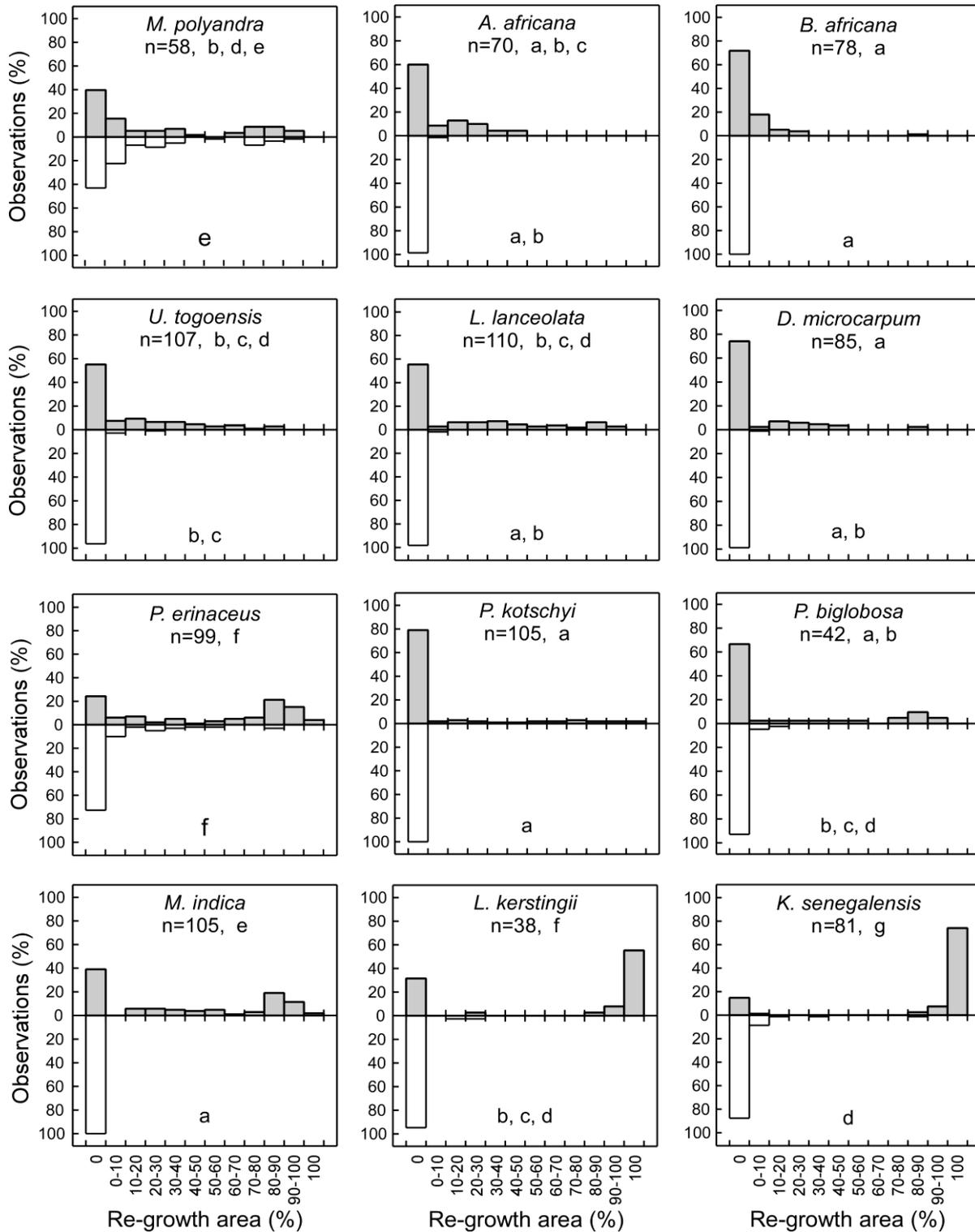
Fig. 2.2: Frequency histograms summarizing the sheet growth of 12 medicinal tree species during two years following bark harvesting. Grey boxes are for re-growth observed after partial bark harvesting, white boxes are for re-growth after total bark harvesting (both techniques were used on each tree). Identical small letters indicate species with no significant difference in sheet growth at the $P \leq 0.05$ confidence level (proportional-odds logit model on score levels, see Table 2.2).
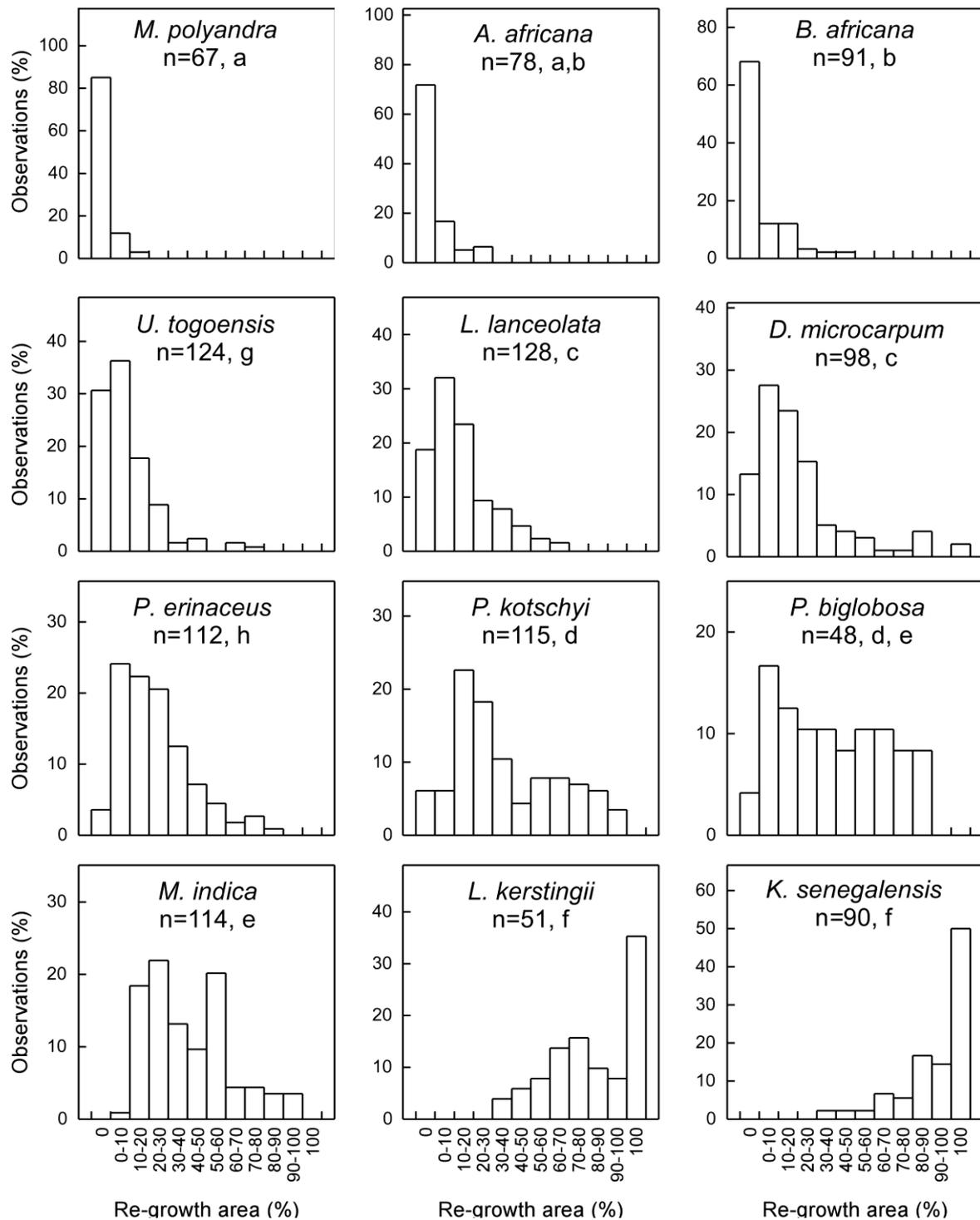
Fig. 2.3: Frequency histograms summarizing the edge growth of 12 medicinal tree species during two years following total bark harvesting. Identical small letters indicate species with no significant difference in edge growth at the $P \leq 0.05$ confidence level (proportional-odds logit model on score levels, see Table 2.2).

INTENSITY OF INSECT ATTACKS

The number of insect holes was clearly species-dependent (Fig. 2.4). *D. microcarpum* and *P. biglobosa* showed the least resistance to insect attack, while several other species, such as *K. senegalensis*, *P. erinaceus*, *M. polyandra*, and *U. togoensis*, were highly resistant. There was a significant positive effect of the surface of non-regenerated area on the frequency of insect attacks ($P < 0.001$), illustrating that fast recovery prevents large-scale insect damage. The effect of species adjusted for different regeneration rates was highly significant ($P < 0.001$), but there was no significant interaction between regeneration rate and species ($P = 0.100$), illustrating that the relationship between regeneration rate and the number of insect holes was not species-specific. It is interesting to note that both *K. senegalensis* and *L. kerstingii* had very good edge growth, reducing their wound area considerably, but their susceptibility to insect attack was quite different: *K. senegalensis* was resistant but *L. kerstingii* was highly susceptible to insect attack. The damage inflicted by insects may weaken the stability of trees, and eventually trees may crack. Over a period of two years, 17.3% of all *L. kerstingii* and 6.8% of all *P. biglobosa* were broken following insect attacks, while no *D. microcarpum*, *B. africana* or *A. africana* trees died from insect attack. This may be explained by the fact that insect holes of *L. kerstingii* and *P. biglobosa* were bigger than those of other species.
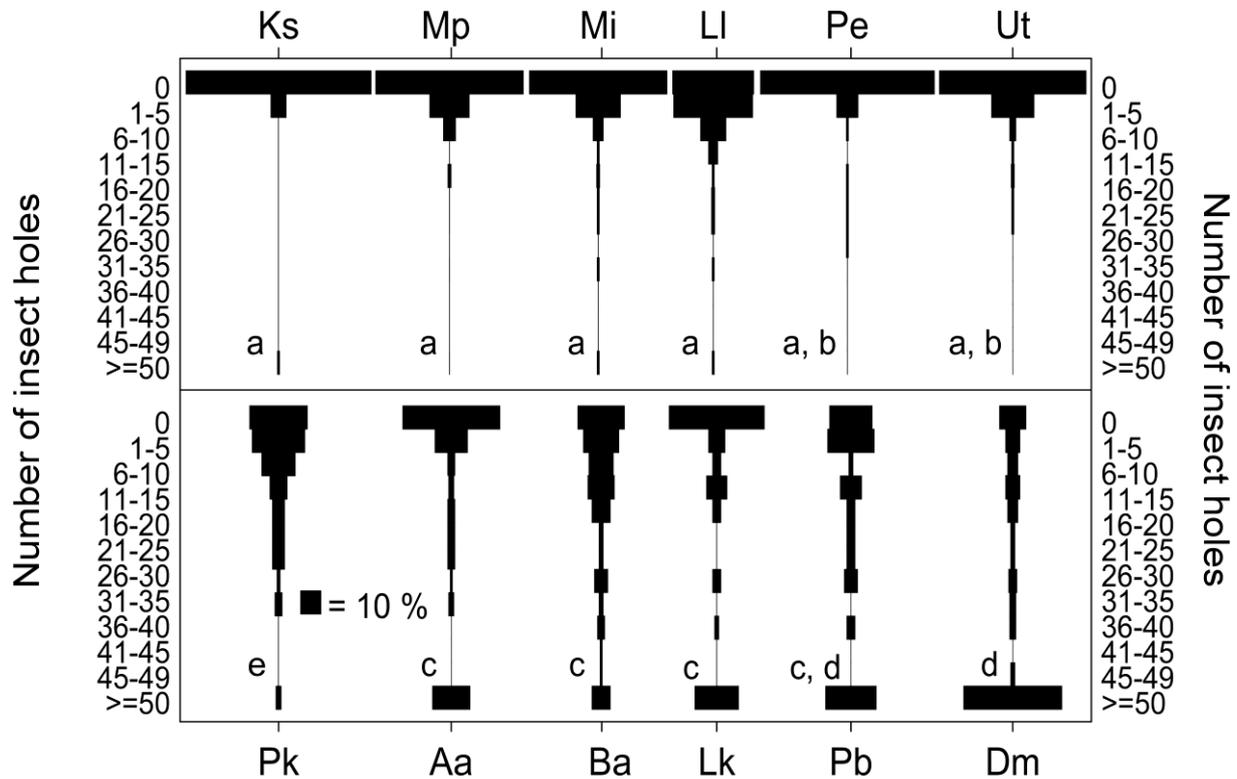
Fig. 2.4: Frequency histograms summarizing the susceptibility of the 12 medicinal tree species to insect attack during two years following total bark harvesting. Aa, *Afzelia africana*; Ba, *Burkea africana*; Dm, *Detarium microcarpum*; Ks, *Khaya senegalensis*; Lk, *Lannea kerstingii;* Ll, *Lophira lanceolata*; Mi, *Mangifera indica*; Mp, *Maranthes polyandra*; Pb, *Parkia biglobosa*; Pe, *Pterocarpus erinaceus*; Pk, *Pseudocedrela kotschyi*; Ut, *Uapaca togoensis*. Identical small letters indicate species with no significant difference in edge growth at the $P \leq 0.05$ confidence level (GLM with species effect adjusted for different regeneration rates).

RESPONSE TO BARK HARVESTING IN TERMS OF VEGETATIVE GROWTH

The development of agony shoots around the wound in response to bark harvesting was largely dependent on species ($P < 0.001$). In this study, only a few trees developed agony shoots (Fig. 2.5). *M. polyandra* presented a slightly greater ability to develop agony shoots than the other species, except for *A. africana*. For the other nine species, only $1 - 13.6\%$ of trees had developed agony shoots by the end of the observation period. No agony shoot was observed around the wound area of *L. lanceolata*. When a tree did develop agony shoots, there were usually only one or two, although *P. biglobosa* and *U. togoensis* produced a mean of 2.5 shoots per tree.

We noticed that *U. togoensis* produced roots around the wound area but we did not investigate this phenomenon further.
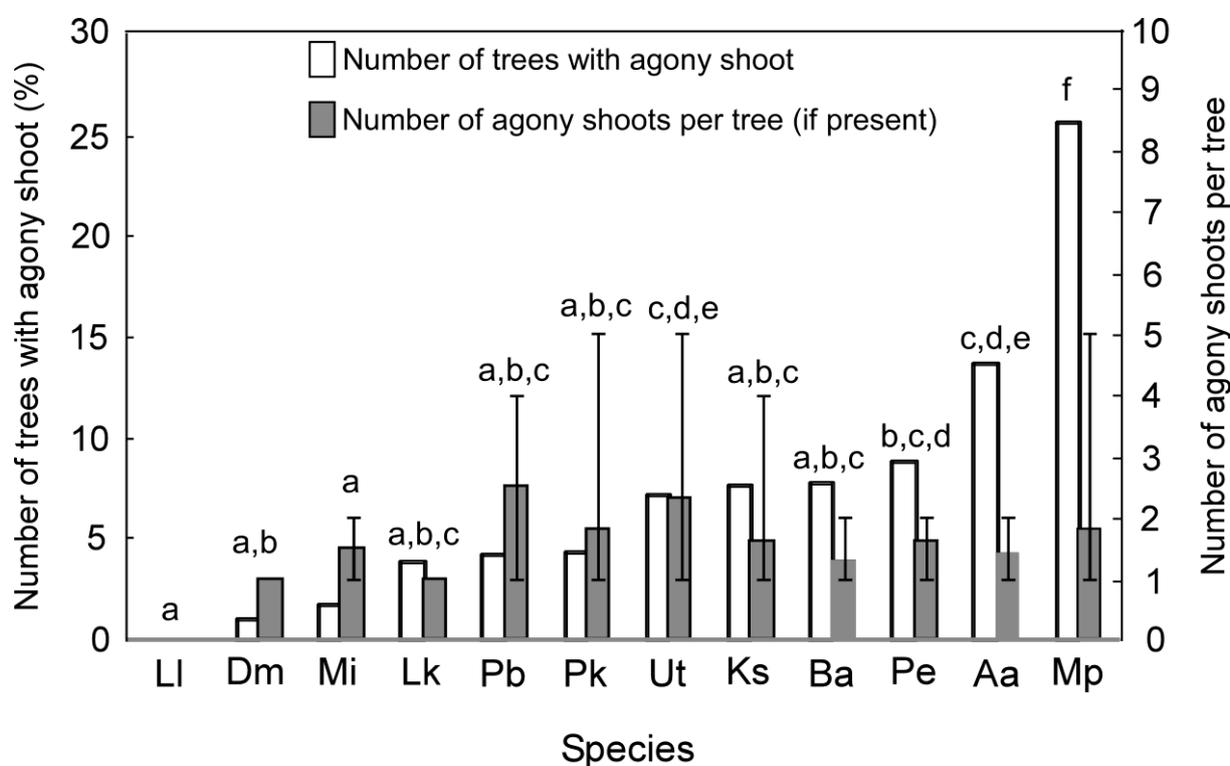
Fig. 2.5: Vegetative growth of the 12 medicinal tree species in response to bark harvesting. The percentage of trees with shoots and mean (± range) of the number of agony shoots/tree with shoots are given. Identical small letters indicate species with no significant difference in edge growth at the $P \leq 0.05$ confidence level. See Fig. 2.4 for abbreviations.

Discussion

The results of this study confirmed the hypothesis that tree response to bark harvesting is species-specific. However, over a period of two years after total bark harvesting, complete bark re-growth was rarely achieved, except for some *K. senegalensis* and *L. kerstingii* trees. Despite some variability among the species tested, it was clear that a harvesting technique based on total bark removal did not favour sheet growth. These findings are consistent with

the results of other studies of different tree species in southern Africa. According to Vermeulen & Geldenhuys (unpublished data), only *Ilex mitis* and *P. africana* had good to poor sheet growth (11 – 60% of re-growth); sheet growth was poor to absent for the four other species studied. Geldenhuys *et al.* (2007) found that, among 22 species harvested, five had good to very good sheet growth, five had poor sheet growth, and the 12 remaining species showed no sheet growth. Guedje (2002)observed that bark wounding in *Garcinia lucida* was not followed by any sheet growth. Similarly, Mariot *et al.* (2007) studied bark harvesting from *Drimys brasiliensis* in southern Brazil and reported sheet growth to be almost non-existent.

Several experiments showed that the most important factor for successful recovery is the humidity of the exposed surface immediately after the wounding (Zimmermann & Brown 1971; Li *et al.* 1982; Neely 1988; McDougall & Blanchette 1996; Stobbe *et al.* 2002; Mwange *et al.* 2003; Du *et al.* 2006). For this reason, those researchers covered the experimental wounds with plastic sheets to obtain significant re-growth. However, applying this technique in the wild is difficult. Consequently, we chose to harvest bark only partially, leaving a thin layer of bark and the cambium on the trunk. Our results demonstrate the protective effect of the remaining bark layer for promoting sheet growth. Compared to total bark removal, this technique increased the percentage of sheet growth significantly for each species studied (Fig. 2.2). However, a large variability in recovery was found among species: only 4 species, *K. senegalensis*, *L. kerstingii*, *P. erinaceus*, and *M. indica*, out of 12 showed a good recovery rate (> 40%). Vermeulen & Geldenhuys (unpublished data) also studied the effect of leaving a thin layer of bark and the cambium in three species, and all of them showed a wound recovery rate of 50 – 80% of the wound surface, whereas none or poor recovery occurred when the bark and the cambium were removed completely .

Several studies have reported bark regeneration starting from the edge of the wound (e.g. Vermeulen & Geldenhuys, unpublished data). Full recovery has been reported for *Betula alleghaniensis* (Shigo 1986), *Prunus africana*, *Warburgia salutaris* and *Ficus natalensis* (Cunningham & Mbenkum 1993), *G. lucida* (Guedje 2002) and *Ocotea bullata*, *I. mitis* and *P. africana* (Vermeulen & Geldenhuys, unpublished data). In this study, however, only *K. senegalensis* and *L. kerstingii* were able to close the wound completely over the two year follow-up period. Our results for *K. senegalensis* did not corroborate the findings reported by Gaoue & Ticktin (2007), who noticed that, in most cases, less than half the wound area was recovered in this species. Our study found that *K. senegalensis* had an average recovery rate of 88.8% of the wound.

In the 12 species studied, we found a negative relationship between the number of insect holes and recovery rate. In this respect, our observations agree with those of Geldenhuys *et al.* (2007) who found that the level of infestation was greater in species that showed none or slow wound recovery. Our study showed susceptibility to insect attack depends on species, independent of regeneration rate. Amongst the 12 species in our study, *D. microcarpum, P. biglobosa*, *L. kerstingii*, *B. africana* and *A. africana* were susceptible to insect attack. One of the most severe effects of wood-boring insects is the failure of the tree at wound level due to large galleries dug deep inside the wood, which was observed for 17.3% of the *L. kerstingii* individuals and, to a lesser extent, for *P. biglobosa*, but only 6.8% of individual stems of that species broke. The impact of insects on *D. microcarpum*, *B. africana* and *A. africana* was limited to the presence of numerous very small holes on the wound surface. The insect holes also facilitated the entry of fungi, which further weakens the wood. Sealant can be applied to the affected area with the aim of limiting the impact of fungi (Botha *et al.* 2004b).

Besides bark recovery, some of the harvested trees produced new roots or and/or shoots (Guedje 2002). If the main trunk dies, the production of new shoots becomes an important survival mechanism. We observed this phenomenon for two *B. africana* trees: the main trunk died but agony shoots, developed beneath the wound, were alive and full of leaves. Burke

(2006) observed the strong ability of *B. africana* to produce coppice shoots. Geldenhuys *et al.* (2007) suggested that the ability of a species to develop agony shoots around the wound after bark harvesting is related to the ability of that species to produce coppice shoots. Of the species in this study, only *D. microcarpum* has been studied for its ability to coppice (Sawadogo *et al.* 2002; Ky-Dembele *et al.* 2007) or to re-sprout (Rietkerk *et al.* 1998; Sawadogo *et al.* 2002). In this study, only one out of 82 *D. microcarpum* trees developed agony shoots. *M. polyandra* showed the greatest ability to develop agony shoots, and it would be useful to test its coppicing ability. Luoga *et al.* (2004) studied the re-sprouting response of 44 species of East African miombo (African savanna) trees and reported the different factors influencing coppicing effectiveness, including the presence or absence of large herbivores and/or fire, season of cutting, site characteristics and species-specific characteristics. Coppices are a potential source of medicinal bark that could be optimized through active coppice management (Vermeulen 2006). Better knowledge of the complex coppicing response of individual tree species would help in the design of specific strategies for sustainable management of woodland containing medicinal tree species (Abbot & Homewood 1999; Bond & Rathogwa 2000; Geldenhuys 2004; Kaschula *et al.* 2005; Neke *et al.* 2006; Ky-Dembele *et al.* 2007).

Our study has several implications for tree management. Harvesting bark requires species-specific techniques to make it sustainable. Sustainable harvesting must take into account the following species-specific factors: (i) the regeneration capacity (edge and/or sheet growth), which may allow for a second harvest; (ii) the susceptibility to insect attack, which may require additional protection measures; (iii) the capacity to develop agony shoots, which may enable the tree to produce coppice shoots. Depending on these factors, two broad management strategies exist: (i) management of tree species in existing natural forests; and (ii) development of alternative resources of medicinal plants outside the forest. Harvesting trees in the wild may include strip harvesting and full-tree harvesting (harvesting of all utilizable bark from the trunk and branches of fallen trees). The latter is bark harvesting as a by-product of timber harvesting and coppice management (Vermeulen 2006). Harvesting trees from an alternative resource includes: establishing stands of the target species in open areas (clearing) and/or forest expansion at the forest edge (Geldenhuys & Delvaux 2002); and harvesting leaves instead of bark. It has been suggested that leaves could be used instead of bark for medicinal purposes, and studies are underway in South Africa to compare the chemical composition of bark and leaves from the same tree (Zschocke *et al.* 2000a; Zschocke *et al.* 2000b; Drewes *et al.* 2001). These methods of increasing bark availability would avoid over-exploitation of tree species in natural forests.

Following the methodology developed by Vermeulen (2006), our results provide the elements necessary to define a strategy that can help forest managers to select the most appropriate bark-harvesting system for different medicinal tree species. The first step involves categorizing species according to their ability to close the wound after bark has been removed, resistance to insect attack, and the ability to develop agony shoots (Table 2.2 and Fig. 2.6). The second step is to choose the appropriate harvest option depending on whether trees recover after bark harvesting: (i) strip harvesting for species with very good re-growth (level 4); or (ii) full-tree harvesting for species with none or little wound closure after bark harvesting (level 1-2-3). The third step is to determine how to manage the full tree harvesting technique according to species characteristics (e.g. through collaboration with a timber company, or via coppice management) and to determine the appropriate alternative solutions (stand establishment, harvesting leaves instead of bark, etc.).
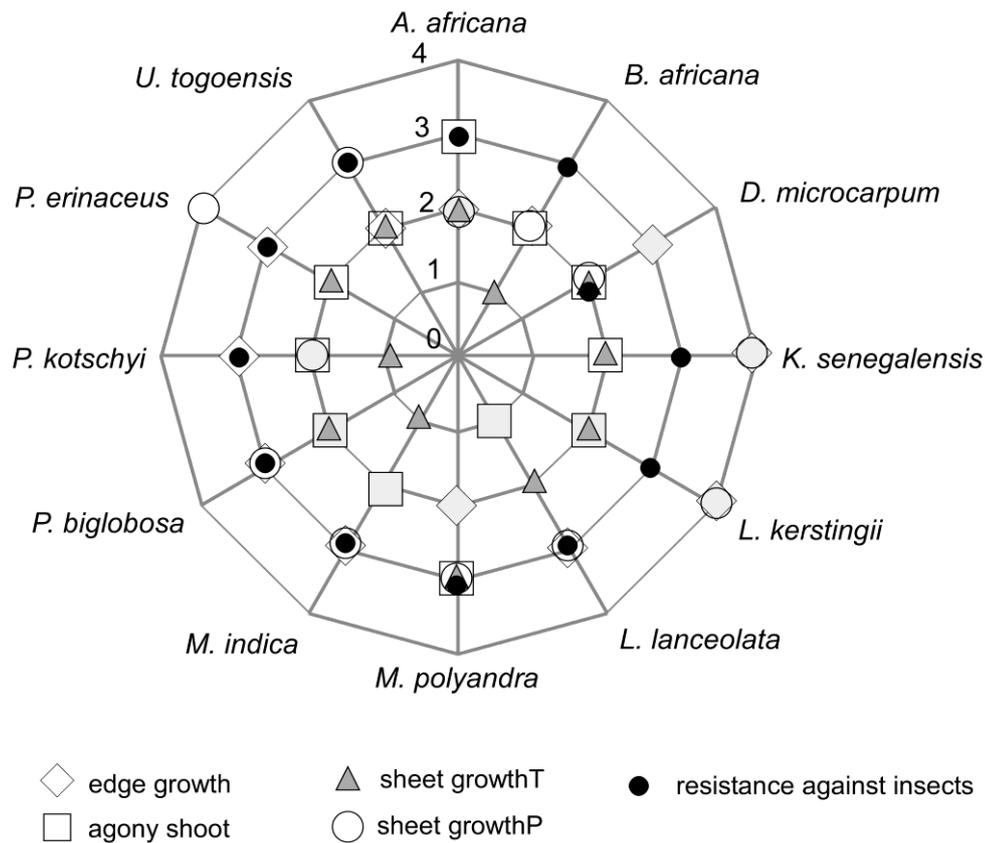
Fig. 2.6: The response scores of the 12 medicinal tree species two years after bark harvesting. See Table 2.2 for further explanation of the variable responses and score levels.

Figure 2.7 gives an overview of the management techniques appropriate, on the basis of our findings, for the 12 species assessed in this study. Bark can be sustainably harvested from *K. senegalensis* and *L. kerstingii*. As the partial bark removal technique allowed a better sheet growth, it could be useful for both species to cover the wound with plastic immediately after harvesting, but *L. kerstingii* must be protected from insect attack. The other 10 species did not exhibit a level of bark regeneration that would allow for a sustainable harvest. We therefore suggest full tree harvesting for these species. This can be done in different ways, depending on the species. Harvesters looking for bark of *A. africana*, *K. senegalensis*, *P. kotschyi* and *P. erinaceus* may approach logging companies that are used to felling large numbers of these species. Bark can be removed without detriment to the wood quality when the tree is cut for timber. *B. africana*, *D. microcarpum* and *M. polyandra* may be cut at 1 m above ground in order to favor coppice shoot development. *M. indica* and *P. biglobosa* should be planted on the forest edge, because they are light-tolerant species and they will be protected by local human populations who appreciate their fruits. For *L. lanceolata*, leaves could be harvested instead of bark. The chemical composition of the leaves and bark of *L. lanceolata* have been analyzed (Pegnyemb *et al.* 1998) but further research is needed to determine the similarity. Thus *L. lanceolata* could be managed as a kind of tea plantation, allowing faster, easier and more frequent harvesting. The results of this study suggest that *U. togoensis* could be used for coppice management. Very little information is available for *U. togoensis*, and it will be

interesting to determine the concentration of the active component for medicinal use in the leaves and bark of this species.
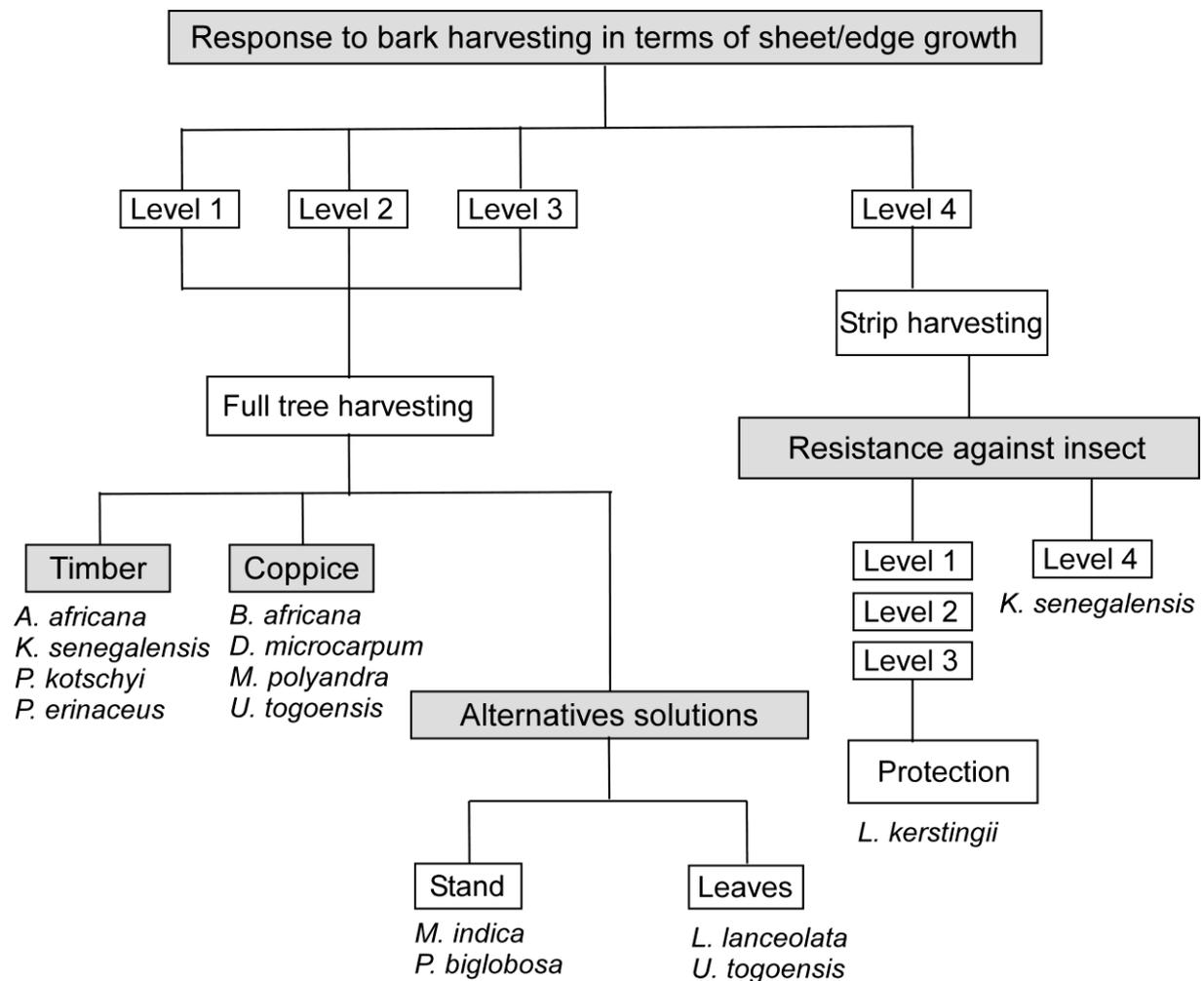
Fig. 2.7: The proposed management strategy for the 12 tree species in this study. The schema illustrates the successive steps needed to provide a decision strategy with the aim of selecting the most appropriate harvesting system for each species. See Table 2.2 for description of levels 1-4.

Acknowledgements

CHAPTER 3

Influence of season, stem diameter and intensity of debarking on survival and bark recovery rate of 12 medicinal tree species, Benin

Submitted to Biological Conservation

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Summary

1. Introduction. The lack of ecological data concerning the responses of tree species to bark harvesting often leads to the overexploitation of medicinal tree species, threatening an essential source of medicines for primary health care of rural populations. This study investigated the ecological consequences of various bark harvesting techniques for 12 tree species at the individual level.

2. Methods. The experimental layout was similar for the 12 species. Trees were debarked following a combination of three factors: (i) season of bark harvesting (during the dry season or during the rainy season), (ii) size class of the tree (three stem diameter classes) and (iii) intensity of debarking (seven intensities ranging from 20% to 100% of trunk circumference debarked). Measurements of edge growth and survival were taken every six months during two years.

3. Main results. Ring-barking (100% of trunk debarked) did not allow the sustainable exploitation of any species, while all trees with 75% of debarked circumference remained alive and produced edge growth. Whatever the bark harvesting technique, for 5 out of the 12 species the bark recovery rate was below 1 cm/year, rendering the wound closure very unlikely. Five species showed good to very good bark recovery rates (> 7 cm/y) and for each species the best combination of factors (season, d.b.h. and intensity) was determined to obtain the highest edge growth.

4. Conclusion. This experimental bark stripping demonstrated the complexity of the management due to the variety of factors influencing bark re-growth. Nevertheless, species-specific results on tree response to bark harvesting can be obtained relatively quickly and allow assessing the best harvest options. Moreover studying the patterns of bark recovery rate is a pertinent management tool to determine for each species the necessary delay to close a specific wound area.

Key-word: Africa, bark stripping, decision tool, medicinal tree, pattern of recovery, ring-barking, season, survival

Introduction

With the recent rise in concern about sustainable management of medicinal plants, there is a need for scientists, economists, sociologists and jurists to generate and use explanatory and quantitative data on harvested species that are currently under exploitation in forest and plantation to define sustainable management strategies and avoid species extinction. Harvesting non-timber forest products (NTFPs), including medicinal plants, often alters the rate of survival, growth and reproduction of harvested individuals (Ticktin 2004; Gaoue & Ticktin 2007). Yet, for many species, the ecological impacts of harvesting, whatever the part that is harvested, are unknown, and this lack of knowledge hinders the identification of sustainable harvesting levels or methods (Hall & Bawa 1993; Grace *et al.* 2002; Ticktin 2004; Ghimire *et al.* 2005; McGeoch *et al.* 2008). Thus, the paucity of ecological knowledge about medicinal plants is a serious problem for resource managers (McGeoch *et al.* 2008). Non-sustainable harvesting not only threatens the survival of valuable medicinal plant species but also the livelihoods of communities that depend on them (Botha *et al.* 2004a; Hamilton 2004; van Andel & Havinga 2008). Moreover, an excessive extraction of forest products is likely to impact negatively on the dynamics of individuals and population of the harvested species, and alter community structure (e.g. Geldenhuys & Van der Merwe 1988; Cunningham & Mbenkum 1993; Stewart 2003; Fashing 2004; Siebert 2004; Ticktin 2004; Ticktin & Nantel 2004; Guedje *et al.* 2007; Ndanyalasi *et al.* 2007; Gaoue & Ticktin 2008).

Tree bark provides the protection against external attack and desiccation and plays a key role in the transport of water and nutrients from leaves to roots through the phloem tissues. Bark removal induces internal stress and may lead to progressive or instant death depending on the extent of harvest. Ring barking, by completely removing a strip of bark around a tree's outer circumference may lead to more or less immediate tree death. However some species may survive ring barking: e.g. cork oak (*Quercus suber*), *Eucommia ulmoides* (Li *et al.* 1982; Li & Cui 1988), *Prunus africana*, *Warburgia salutaris*, *Ficus natalensis* (Cunningham & Mbenkum 1993) or *Carapa procera* (Delvaux, unpublished data). These results have proved that it is important to test several bark harvesting treatments (including ring barking) to determine the harvest limit. Few studies were already undertaken to estimate the maximum sustainable harvest rate e.g. leaves and ramet of *Aechmea magdalenae* (Ticktin *et al.* 2002), rhizomes of *Nardostachys grandiflora* and *Neopicrorhiza scrophulariiflora* (Ghimire *et al.* 2005), rattan of *Calamus zollingeri* (Siebert 2004), bark of *Garcinia lucida* (Guedje *et al.* 2007). Defining a bark maximum sustainable harvesting limit for harvested species is necessary to ensure the persistence of individuals and populations.

One of the major problems a debarked tree faces is rapid and sufficient bark recovery to close the wound and provide the optimum protection. In a recent study, Delvaux *et al.* (2009) were able to show that species respond to bark harvesting in various ways: e.g. bark re-growth (from edge or/and from sheet), development of agnoy shoots (= vegetative shoots developing around a wound in response to wounding). Bark and wood recovery after debarking involve many intrinsic changes (e.g. Benayoun *et al.* 1975; Rademacher *et al.* 1984; Oven & Torelli 1994; Schmitt *et al.* 1997; Novitskaya 1998; Frankenstein *et al.* 2005; Pang *et al.* 2008). Although there is a significant interest in the bark harvesting rates (e.g. Cunningham & Mbenkum 1993; Stewart 2003; Vermeulen 2006; Geldenhuys *et al.* 2007; Delvaux *et al.* 2009) our knowledge of the sustainable harvesting cycle and time elapsed between harvesting events is still limited for most tree species. Only the temporal pattern of bark recovery of *Quercus suber* was already studied and it is known that cork can only be harvested every 9-15 years and the Portuguese legislation imposes a minimum of nine years (Moreira *et al.* 2009). We hypothesize that the bark recovery rates and thus their harvesting frequency is species-dependent.

To acknowledge consequences of tree bark harvesting and to give appropriate recommendations for a sustainable management, we hypothesized that seasons, stem diameter at breast height (d.b.h.) and intensity of harvesting may influence bark recovery of 12 medicinal tree species. Thus the implication of the output of this study would enable the formulation of specific management strategies for each species. Specifically we addressed the following questions:

1) How do maximum debarking rates vary between species under different intensities and timing of bark harvesting (dry vs. rainy season)?
2) For each species, what is the delay needed to close the wound completely after bark harvesting?
3) What are the effects of harvest seasons (i.e. dry or rainy season), size of the tree (d.b.h.), and various harvest treatments on a specie's ability to re-grow new bark?

Methods

STUDY AREA AND SPECIES

This study was carried out in the Forêt Classée des Monts Kouffé in central Benin (8°30' - 8°52' N, 1°40' - 2°27' E). 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 located in the Sudano-Guinean region (Adomou *et al.* 2007). Study sites were selected in a *Isoberlinia* spp woodland on ferruginous soils. Like most protected areas in Benin, the Forêt Classée des Monts Kouffé is somewhat degraded due particularly to encroachment for agriculture. Our sites were located away from farms. The tropical rainy season during May to October has a unimodal regime. Mean monthly rainfall during the study period in 2004, 2005 and 2006 were 138 mm, 189 mm and 165 mm respectively (ranging from 21.5 mm to 306.2 mm). The mean monthly rainfall in the dry season was 15 mm in 2006 (ranging from 2.1 mm to 42.5 mm). The annual temperature ranged from 25°C to 34°C and they were similar 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. The frequent and regular dry season bush fire put all living organisms in the study area under stress. Based on ethnobotanical survey in the region (Bockx 2004), we selected 12 medicinal tree species (Table 3.1) known to be debarked for primary health care by the local communities.

EXPERIMENTAL DESIGN

We conducted our experimental debarking of 20 sites in the dry season and 18 sites in the rainy season. For each species only healthy trees (no previous bark harvesting) were selected for the experiment. On each individual, bark was harvesed from trunk at 1 m stem height. The wound was rectangular in shape with the vertical side 30 cm long and the horizontal width varying depending on the applied intensity (see below). To assess the effect of harvesting season trees of each species were harvested both during the dry season (February and March) and during the rainy season (September and October) in 2004. We defined three classes of diameter at breast height (d.b.h.) at which debarking occurred: 10-20 cm (d.b.h.1), 21-30 cm (d.b.h.2) and > 30 cm (d.b.h.3). Seven intensities (I) of bark harvesting were implemented to cover the different harvesting practices (expressed in percentage of the circumference of the debarked tree): 20% (I1), 2 x 10% (I2), 50% (I3), 2 x 25% (I4), 20% (I5), 75% (I6) and 100% (I7). For Intensities I2 and I4, bark was harvested on both sides (east and west) of the trunk. For intensity I5, a square was harvested instead of rectangle. Each intensity was applied for

Table 3.1: The 12 tree species used in this study. The number of individual trees observed (N) and the range of diameter at breast height (d.b.h.) values are given

Species	Family	N	d.b.h. (measured) (cm)
Afzelia africana Sm.	Fabaceae (C)	68	15.6-41.7
Burkea africana Hook.	Fabaceae (C)	78	11.6-44.0
Detarium microcarpum Guill. & Perr.	Fabaceae (C)	82	13.5-45.0
Khaya senegalensis (Desv.) A. Juss.	Meliaceae	73	12.0-36.4
Lannea kerstingii Engl. & K. Krause	Anacardiaceae	48	17.0-44.9
Lophira lanceolata Van Tiegh. ex Keay	Ochnaceae	102	14.9-36.4
Mangifera indica L.	Anacardiaceae	86	12.2-47.2
Maranthes polyandra (Benth.) Prance	Chrysobalanaceae	53	12.8-35.1
Parkia biglobosa (Jacq.) R. Br. ex G. Don	Fabaceae (M)	44	14.0-49.5
Pseudocedrela kotschyi (Schweinf.) Harms	Meliaceae	93	13.3-40.4
Pterocarpus erinaceus Poir.	Fabaceae (P)	96	13.5-40.5
Uapaca togoensis Pax	Euphorbiaceae	102	12.3-48.2

(C): Caesalpinioideae; (M): Mimosoideae; (P): Papilionoideae

each diameter class except for I6 and I7 which were applied only for d.b.h.2. We marked each selected trees with coloured plastic ribbon and numbered aluminum tag. Bark was harvested from a total of 925 trees. The number of individual trees per species is given in Table 3.1. The reasons for differences in the number of trees per species were: (i) difficulty in finding trees with an appropriate diameter according to the species morphology. In the wild, it is rare to find examples of *Burkea africana*, *Detarium microcarpum* or *Maranthes polyandra* with a d.b.h. > 30 cm. (ii) Some species (*Afzelia africana*, *Khaya senegalensis*, *Pseudocedrela kotschyi*, and *Pterocarpsus erinaceus*) had been heavily harvested for timber, and thus, trees with d.b.h. higher > 30 cm were scarce in the study area. (iii) Some species naturally have a sparse distribution (e.g. *Lannea kerstingii*, and *Parkia biglobosa*) and finding a sufficient number of these species would have required excessive travelling and time.

FIELDS MEASUREMENTS

All trees were monitored a month after bark harvesting, and then every six months during the two-year study period. For each tree its survival and extent of bark re-growth were recorded. A tree was considered dead when it lost all its foliage (the species phenology which was monitored throughout the study period) and if there was any sap by making a very small cut in the trunk with a knife. Bark re-growth is defined as the live tissue developing from the edge of the wound. This re-growth is called edge growth. Three horizontal measurements (cm) were made from fixed points drawn on both sides (left and right) of the wound. To calculate the total edge growth (cm), the mean value of these three measurements was added for both sides (left and right). To study the pattern of bark recovery for each species, the bark recovery rate was considered as the amount of new tissues (cm) produced over time.
Sheet growth (i.e. live tissue re-growth on the surface of the wound) was also measured, the state of the crown and noted the presence of agony shoots around the wound.

DATA ANALYSES

To test if species, season and intensity of bark harvesting significantly influenced tree survival after bark harvesting, we used a Generalized linear model with a binomial distribution in R (R Development Core Team 2005). To compare the variation in the different patterns of bark recovery rate within the 12 species, we classified species within four groups based on data recorded every six months during two years. These data corresponded with the measurements of edge-growth expressed in cm. To test the effect of season, tree size and intensities of bark harvesting on the ability of tree species to regenerate the new bark (cm), individual scores were calculated according to 14 levels ranging from 0 to 50 cm with class interval of 4 cm. Ordinal score levels were compared between each factor (season, d.b.h., intensity) for each species by a proportional-odds logit model using a polr procedure in R (R Development Core Team 2005). For this latter test, only species which presented a mean bark recovery rate higher than 4 cm/year (*K. senegalensis*, *L. kerstingii*, *Mangifera indica*, *P. biglobosa* and *P. kotschyi*) were analysed to provide relevant proposals for a sustainable management of bark harvesting.

Results

EFFECTS ON SURVIVAL AND MAXIMUM BARK HARVESTING LIMITS

At the start of this research, a total of 925 trees over 12 species, were bark harvested. Over the two-year study period, 72 of the 925 trees harvested died.
Regardless of seasons and treatments applied, mortality rates varied significantly between the 12 species (Fig. 3.1). *M. indica* was the only species for which all debarked trees remained alive and *A. africana* lost only 2 out of 66 trees. On the contrary, *L. kerstingii* was the most sensitive to bark harvesting with a mortality rate of 17.3%. *Lophira lanceolata*, *P. biglobosa*, *P. kotschyi* and *Uapaca togoensis* had similar mortality rates (Fig. 3.1). The five other species lost relatively few trees.
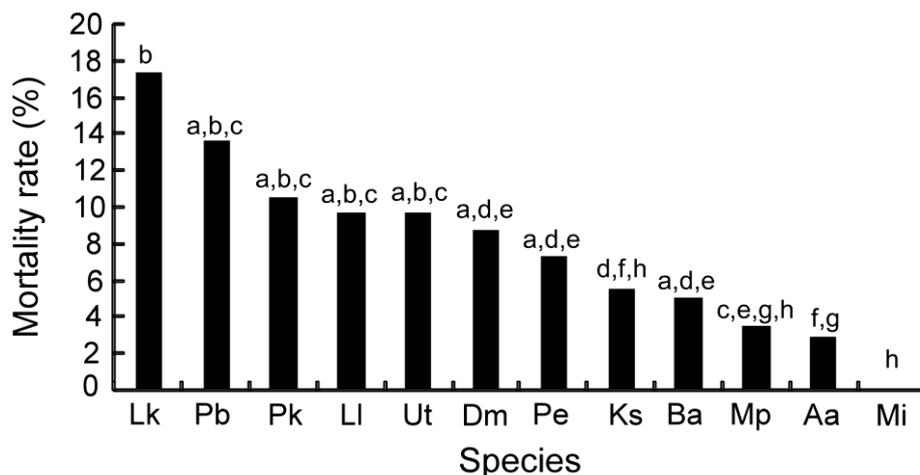
Fig. 3.1: Mortality rate (%) after bark harvesting for 12 medicinal tree species regardless of season and intensity of bark harvesting inflicted. Aa, *Afzelia africana*; Ba, *Burkea africana*; Dm, *Detarium microcarpum*; Ks, *Khaya senegalensis*; Lk, *Lannea kerstingii;* Ll, *Lophira lanceolata*; Mi, *Mangifera indica*; Mp, *Maranthes polyandra*; Pb, *Parkia biglobosa*; Pe, *Pterocarpus erinaceus*; Pk, *Pseudocedrela kotschyi*; Ut, *Uapaca togoensis*. Identical small letters indicate species with no significant difference at the $P \leq 0.05$ confidence level (GLM with a binomial distribution).

The mortality rate of harvested trees was significantly higher when harvesting occurred in the rainy season (69.4% of all dead trees) than in the dry season (30.6% of all dead trees) (GLM; $P = 0.0175$). At species level, *B. africana*, *M. polyandra*, *L. kerstingii* and *P. biglobosa* lost trees only when they were harvested during the rainy season (Fig. 3.1). Season of harvest did not have a significant effect on the mortality rates of *A. africana* and *U. togoensis*. *P. erinaceus*, *L. lanceolata* and *P. kotschyi* trees died in both seasons but they showed a tendency to a better survival when they were harvested in the dry season, the opposite was true for *K. senegalensis* and *D. microcarpum*.

Trees debarked at 100% (I7) were the most affected (Fig. 3.2) and caused the death of 44 trees (60% of dead trees). *A. africana*, *M. polyandra* and *K. senegalensis* suffered mortality only in that intensity. For all species 100% debarking resulted in death of two or more trees per species, except *M. indica* for which all individuals survived. After two years, 75.9% of ring-barked trees had died. For the 12 species, almost all the trees remained alive for the first six months. Between 6 and 18 months, 39 trees died. During the last six months of our experiment, the mortality was very low. The survival rate was low (24.1%) but yet 14 ring-barked trees survived at least two years.

Trees with 50% of trunk circumference debarked (I3) had the second worst survival rate (21% of all dead trees) (Fig. 3.2). Under this treatment (I3), 100% of trees survived for *A. africana*, *B. africana*, *D. microcarpum*, *K. senegalensis*, *M. polyandra* and *M. indica* (Fig. 3.2).
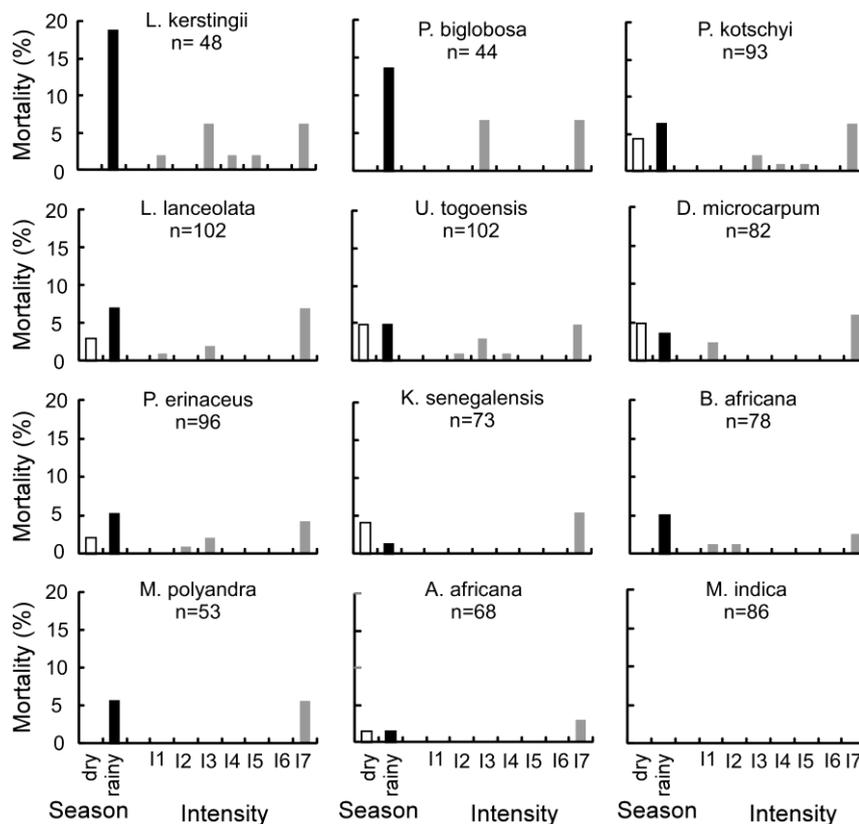
Fig. 3.2: Number of dead trees recorded for 12 medicinal tree species two years after they have been harvested during the dry season (white boxes) and during the rainy season (black boxes) and the number of dead trees recorded for 12 medicinal tree species two years after they have been harvested according to seven different intensities (grey boxes). n = number of trees harvested at the beginning of the experiment. Portion of the trunk debarked: I1= 20% of the trunk circumference, I2= 2 x 10 %, I3= 50%, I4= 2 x 25%, I5= 20% with square shape, I6 = 75 % and I7 = 100%.

After two years, intensity I3 led to the death of only 9.4% i.e. 15 trees out of 167 trees wounded by that intensity. The first dead trees were observed after six months. Most of the trees (11/15) died between 6 and 18 months. During the last six months of observation, only one tree was lost.

Over the two-year study period, intensities I1, I2, I4 and I5 caused the death of only two to five trees and all trees with 75% trunk debarked (I6) remained alive (Fig. 3.2).

PATTERN OF BARK RECOVERY RATES

Bark recovery rates ranged greatly across the 12 species (Fig. 3.3). Average annual bark production varied from zero for *M. polyandra* to 10.8 cm/year for *K. senegalensis*, both harvested during the rainy season. Four groups of bark recovery rates were determined based on the amount of bark produced annually (Fig. 3.3). For group 1, edge growth was very low with a rate of wound closure below 1 cm/year. Moreover there was almost no increase in recovery rate over the 24 months of experiment. Thus, once trees have been harvested the wound remains open. By contrast, *L. kerstingii* and *K. senegalensis*, belonging to group 4, had a high bark recovery increase since the first six months after debarking. With a constantly high increment over time, these species had the best annual bark recovery rate and most of them eventually closed the wound after 24 months. For species of group 3, edge growth really started after a six-month delay. It is particularly true for *M. indica* debarked during the rainy season. Only for *P. biglobosa* harvested during the dry season, 65% of newly formed bark died over the course of the experiment, in the last six months. For this species, the season of harvest substantially influenced the pattern of wound closure.
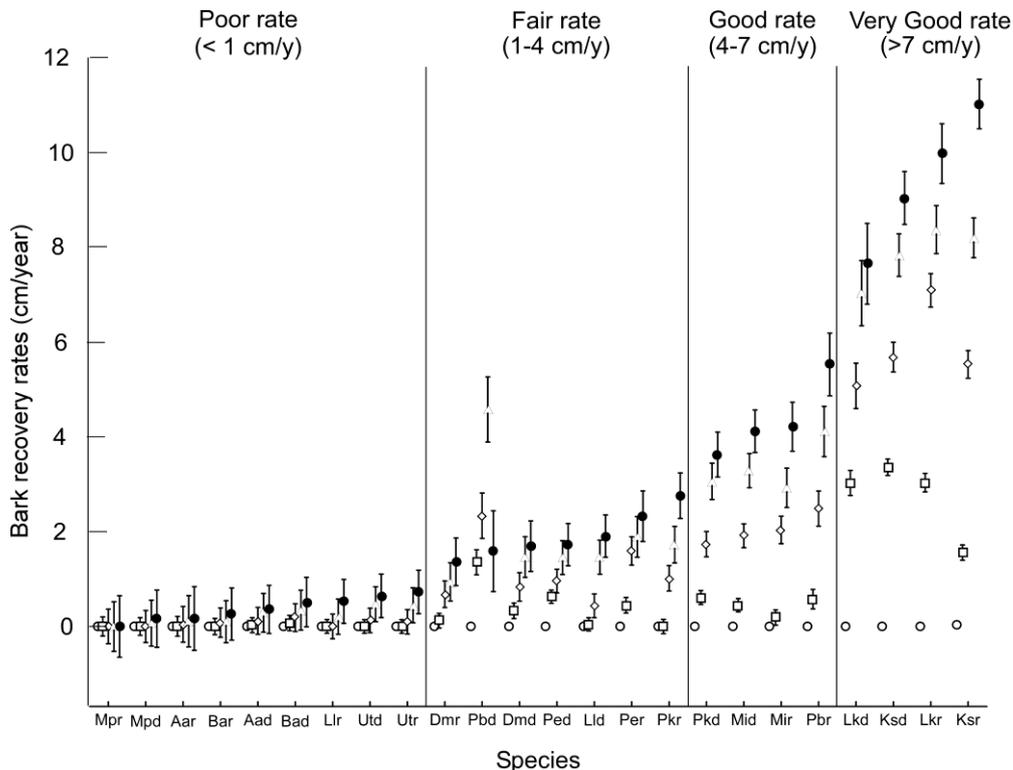
Fig. 3.3: Biannual pattern of bark recovery rates (mean ± SE) belonging to 12 medicinal tree species. Four groups are determined according to the amount of bark produced two years after debarking. See Fig. 3.1 for abbreviations of species. d= species debarked during the dry season and r = species debarked during the rainy season. ○= 1st month, □ = 6th month, ◊ = 12th month, Δ = 18th month, ●= 24th month.

IMPACT OF SEASON, D.B.H. AND INTENSITY OF HARVESTING ON BARK RECOVERY

The season of the bark harvesting (dry vs. rainy season) affected the re-growth of bark across the five selected species (Table 3.2). Bark re-growth of *L. kerstingii* and *P. biglobosa* was significantly higher in the rainy season than in the dry season (polr, $P = 0.019$ and $P = 0.001$ respectively). In contrast to these species, there was no significant difference in seasons of bark harvesting for *K. senegalensis*, *M. indica* and *P. kotschyi* (polr, $P = 0.121$, $P = 0.498$, $P = 0.066$ respectively).

Bark recovery rate was size dependent except for *L. kerstingii* and *M. indica* (Table 3.2). No common trend was observed between three other species. The re-growth of *K. senegalensis* varied significantly across the three d.b.h. classes. *K. senegalensis* trees of large size > 31 cm (d.b.h.3) showed a significant higher bark regeneration than the recovery of trees with smaller size, i.e. d.b.h.2 class and d.b.h.1 class. The opposite was true for *P. biglobosa* where trees belonging to d.b.h.1 class had significantly higher bark recovery rate than trees belonging to d.b.h.2 and d.b.h.3 classes. *P. kotschyi* presented a completely different response pattern. Trees with medium size (21-30 cm d.b.h.) had a faster bark recovery than the other two d.b.h. classes (d.b.h.1 and d.b.h.3) which had a similar regeneration rate.

Bark recovery rate was highly dependent on the amount of harvested bark (Table 3.2). For *K. senegalensis*, *M. indica* and *P. kerstingii*, Intensity I6 (75% trunk debarked) yielded significantly higher bark re-growth after harvest and Intensity I2 (2 x 10% trunk debarked) resulted to a significantly weaker recovery for *K. senegalensis*, *M. indica* and *P. biglobosa*. For *L. kerstingii* and *P. biglobosa* which were not harvested at Intensity I6 (75% trunk debarked), the higher edge growth appeared after the treatment of Intensity I3 (50% trunk debarked).

Table 3.2: Influence of season, size class (d.b.h.) and intensity of debarking on edge-growth (mean±SE, cm/y) during the two years following bark harvesting. Only the five species showing a bark recovery rate higher than 100cm²/year are tested.

	Species				
	K. senegalensis	*L. kerstingii*	*M. indica*	*P. biglobosa*	*P. kotschyi*
Season					
dry season	9.7±0.9 a	7.8±0.9 a	4.1±0.3 a	1.9±0.7 a	3.7±0.4 a
rainy season	11.7±0.8 a	10.2±0.7 a	4.3±0.3 a	6.2±0.5 b	3.1±0.4 a
Size class					
d.b.h.1	9.0±0.9 a	8.8±1.0 a	3.7±0.4 a	6.7±1.0 a	3.1±0.4 a
d.b.h.2	12.2±0.8 b	9.5±1.0 a	4.3±0.3 a	4.7±0.9 a,b	3.7±0.4 b
d.b.h.3	15.8±2.7 c	9.9±1.0 a	4.3±0.3 a	3.7±0.7 b	3.1±0.5 a
Intensity					
I1	10.6±1.2 a,c	8.3±0.8 a	4.8±0.5 a	4.5±0.8 a,b,c,d	2.9±0.6 a
I2	6.8±1.3 b	7.2±1.1 a	3.5±0.4 b	2.7±1.1 a	3.2±0.5 a
I3	13.0±1.2 a,d	12.0±1.7 a,b	4.6±0.5 a	6.9±1.1 b	3.2±0.6 a,b
I4	8.9±1.1 c	6.3±2.1 a	3.8±0.4 a	5.7±1.7 b,c,d	1.7±0.5 a
I5	14.9±1.4 d	11.7±0.9 b	4.5±0.5 a	4.4±1.3 a,c,d	3.2±0.7 a,b
I6	15.2±2.4 a,d	-	3.8±1.0 a	-	5.5±0.9 b

Identical small letters indicate no significant difference at the $P \leq 0.005$ confidence level (proportional-odds logit model on score level, see text). d.b.h.1: 10-20 cm, d.b.h.2: 20-30 cm, d.b.h.3: >30 cm. Portion of the trunk debarked: 30 cm high and I1= 20% of the trunk circumference, I2= 2 x 10 %, I3= 50%, I4= 2 x 25%, I5= 20% with square shape, I6 = 75 %.

Discussion

MAXIMUM BARK HARVESTING LIMITS

Our study showed that when trees were debarked at 75% of their trunk circumference, they could survive at least for the next two years. However 100% trunk debarking killed 75.9% of trees died over the two-year study period, suggesting an unsustainable harvesting rate. *M. indica* was the most resistant species and could thrive after ring-barking for at least two years after harvesting. The contrary was true for *L. kerstingii*, *M. polyandra*, *P. biglobosa* and *P. kotschyi* which lost all their ring-barked trees within the two-year period of experiment. Globally, our study showed that trees were able to survive at least six months after high debarking intensity and then they died successively.

A species may survive ring-barking, if it is able to recover the bark rapidly by producing a surface callus from the wound callus. The surface callus originate from the trunk cambium and/or from dedifferentiation of immature xylem cells (Li & Cui 1983; Stobbe *et al.* 2002). Species such as *Quercus suber* and *Eucommia ulmoides* had the ability to recover the bark easily following ring-barking (Li *et al.* 1982; Li & Cui 1988). The lack of sufficient sheet growth to create a new photosynthates transport structure between leaves and root, may explain the mortality of trees after ring-barking. We observed trees of *K. senegalensis* and *M. polyandra* that produced sheet growth equivalent to 51.9% and 89.6% respectively of total wound surface area, although this bark re-growth was not large enough to close the wound. Moreover this bark regeneration did not survive longer than six months. Gaoue and Tickin (2007), also reported that ring-barked trees of *K. senegalensis* did not survive. Similar results were also reported for *Garcinia lucida* in Cameroon (Guedje *et al.* 2007). Overall, our study confirmed that ring-barking or 100% trunk debarking for our study species is not a sustainable technique. Given the biology of some species, a better alternative would be to cut individuals at 1 m height and then harvest their bark. We expect them to coppice new trunks and generate new individuals over time. Similar coppice management was already proposed by previous studies as a bark harvesting technique on *Garcinia lucida* (Guedje *et al.* 2007), *Ocotea bullata* (Vermeulen 2006) and *A. africana*, *B. africana*, *P. biglobosa* and *P.erinaceus* (Delvaux *et al.* 2009).

The exact reasons why trees were still alive while the transport of water, nutrients between leaves and root were interrupted remain unknown. Thus, based on our study case where 14 trees (24.1% of ring-barked trees) remained alive after ring-barking, it would be very interesting to investigate the post ring-barking survival strategy. In our study site, *M. indica*, *A. africana*, *K. senegalensis*, *P. erinaceus* which showed cases of post ring-barking survival could be interesting study species for this purpose.

PATTERN OF BARK RECOVERY RATES

Our experimental harvesting showed that many species had a slow response to bark removal over the experimental period (Fig. 3.3). Estimates of recovery times are useful to develop sustainable harvesting strategies (Ticktin 2004). There is a need to develop long-term studies to suggest appropriate management (Nakazono *et al.* 2004). Indeed, if the intervals between harvests are short, the density of these trees may diminish to a point where they will be difficult to find or may become locally extinct, resulting in scarcity of bark (Guedje *et al.* 2007). Thus, in the absence of information, management plans would be developed and implemented on the basis of limited ecological data (Nakazono *et al.* 2004; Ticktin 2004; Emanuel *et al.* 2005). The study of patterns of bark recovery rate offers double information. We are able to determine either the time (month, year) needed to close an exact wound area,

or the maximum debarked area that will be closed in the course of an exact delay. For *A. africana*, *B. africana*, *D. microcarpum*, *L. lanceolata*, *M. indica*, *M. polyandra*, *P. biglobosa*, *P. erinaceus*, *P. kerstingii* and *U. togoensis*, the two-year study period was too short to provide specific management prescriptions. Nevertheless, from our biannual survey of bark recovery rate we can project the bark recovery time for each species (Fig. 3.3). In our study, we consider that a bark recovery rate of 7 cm/year is the minimum growth rate necessary to close the wound completely within two years after bark harvesting. This was based on previous results (Delvaux *et al.* 2009) showing that only *K. senegalensis* and *L. kerstingii* (Group 4) trees were able to close the wound within the two-year study period. In contrast, for species with poor bark recovery rate such as *A. africana*, *B. africana*, *M. polyandra*, *U. togoensis* and *L. lanceolata* (Group 1), it is unlikely that they will recover their bark after two years. A similar conclusion was made for *Rapanea melanophloeos* (Vermeulen 2006). The different response between these two extreme groups may be attributed to the variation in the anatomical composition and tissue structure of wood and bark. For instance, closure is best when the cambium "slides" over the wound surface. Consequently, if the cambium turns inward to form a callus roll, the wound may never really close (Shigo 1986). However, this has to be confirmed through detailed study of wood production after wounding. Fair rates of bark recovery observed for *D. microcarpum*, *P. erinaceus* and *L. lanceolata* (Group 2) may be explained by the wholly loss of leaves during 3-4 months from October to February. Whatever the season of debarking, the recovery occurring during the dry season (i.e. loss of leaves) showed a lower increment than during the rainy season (i.e. fully leaved). The amount of photosynthates produced by a tree fluctuates depending on the different events in its life. When a tree sheds its leaves, inducing cambial dormancy (Devineau 1999; Schongart *et al.* 2002), very little photosynthesis is taking place, and the tree has to rely on reserve energy from the previous year. When new leaves appear, they start producing more photosynthetates, but most of this energy is being used by the process of leaf formation in the early part of the period, then radial stem growth occurs within a few weeks following full leaf expansion. Consequently for deciduous species less energy is available to heal a wound over a one-year cycle, which explains that these species may need five or more years to close the wound. The loss of 65% of newly formed bark for some *P. biglobosa* may be explained by a combination of several factors. *P. biglobosa* is wholly or partially leafless while flowering and appears to be sensitive to environmental factors such as drought (Bayala *et al.* 2008). Moreover, in this part of the study area bush fires are a seasonal stress for the trees. In our study, species belonging to Group 2 need at least five years to close the wound. With similar intensities as applied in this study, species belonging to Group 3 (4-7 cm/y) would be able to close the wound within four years. In our study, *K. senegalensis* and *L. kerstingii* are the only two species presenting very good bark recovery rates (Group 4). The deeper root system of *K. senegalensis* trees (Ouedraogo-Koné *et al.* 2007), which may give better access to soil moisture and nutrients, and its deciduous phenological status (the species sheds its leaves during the dry season but they are replaced as they fall) (Devineau 1999) may explain why this species keeps a high bark recovery rates throughout the year. Moreover *K. senegalensis* is fast growing and light demanding (Nikles *et al.* 2008). These intrinsic characteristics may therefore partly explain its resilience. In contrast, to the best of our knowledge *L. kerstingii* remained an enigma for us. It is a pronounced-deciduous species completely shedding leaves from November to February thus it is short of energy, however the bark recovery rate was similar throughout the year. Moreover the bark production rate is the second best across all our studied species. Hence, the intra-specific and inter-specific differences measured over this two-year study also indicated the influence of a genetic factor favouring or preventing wound closure.

The similarity across the 12 species is that bark recovery rate was equal to zero during the first month after debarking. Although at an anatomical level healing reactions start immediately after wounding inflicted by bark harvesting (e.g. Schmitt & Liese 1993; Stobbe *et al.* 2002), no edge growth was usually measured on any tree over the first month after bark harvesting. Indeed, during this period the tree establishes boundaries within the wood present at the time of wounding to restrict the spread of microorganisms, which is vital for the protection of vascular, storage and meristematic tissues in wounded living trees. This well-known phenomenon is called compartmentalization and it results in production of tyloses into the lumen of vessels and accumulation of phenolic compounds in the parenchyma surrounding the wound (Pearce & Holloway 1984; Shigo 1984a; Schmitt & Liese 1994; Clerivet *et al.* 2000; Sun *et al.* 2006). Moreover an abnormal parenchymatic cell proliferation occurs to form the wound callus (Grünwald *et al.* 2002; Frankenstein *et al.* 2005). The development of a wound callus enables early formation of protective ligno-suberized layer and wound periderm, which are necessary before dedifferentiation of new cambium, initiating the wound closure process (e.g. Oven *et al.* 1999; Grünwald *et al.* 2002; Frankenstein *et al.* 2005). These mechanisms occur during the first and second months after wounding (Schmitt & Liese 1993; Oven & Torelli 1994), thus few new tissues are produced during this period.

INFLUENCE OF SEASON, D.B.H. AND TREATMENT

K. senegalensis, *L. kerstingii*, *M. indica*, *P. biglobosa* and *P. kotschyi* were selected because of their good to very good rates of bark recovery (Group 3 and Group 4) and thus their potential ability to support a sustainable bark harvesting. Aiming at giving pertinent and appropriate management advice, a broad guideline is provided for these species in terms of season and intensity of harvesting and tree size.

Our results illustrate that bark harvesting during the rainy season led to a better bark recovery for *L. kerstingii* and *P. biglobosa*. In contrast *K. senegalensis*, *M. indica* and *P. kotschyi* showed similar bark recovery irrespective of the harvesting season. The humidity of the exposed wound is the most important factor to allow the start of the bark recovery process (Li *et al.* 1982; Neely 1988; McDougall & Blanchette 1996; Stobbe *et al.* 2002; Mwange *et al.* 2003). In woodlands where the canopy is not closed and tree trunks receive the sun rays, the external humidity affects them only during the rainy season. Moreover during this season no fire occurs. Nevertheless, the variety of factors influencing the trees' response to season of bark stripping and the variable responses from different trees species, do not allow for easy interpretation of experimental results that could influence harvest prescriptions (Vermeulen 2009).

It is interesting to note that the size of the tree did not have an effect on the bark recovery rate for *L. kerstingii* and *M. indica*. The contrary was true for *K. senegalensis*, *P. biglobosa* and *P. kotschyi* but the size class of trees showing the best bark recovery was different for each species: > 30 cm, 10-20 cm and 21-30 cm respectively. This confirmed observations obtained by Gaoue and Ticktin (2007) who showed that local people harvested more bark from *K. senegalensis* trees between 35 and 95 d.b.h. than from trees between 5 and 39 cm d.b.h.. Vermeulen (2006) also found that smaller trees of *Ocotea bullata*, *Curtisia dentata* and *Rapanea melanophloeos* were more affected by an experimental bark harvesting in Southern Cape forest, South Africa. This latter experimental work confirmed inventories carried out in KwaZulu- Natal forest, South Africa, where populations of these three species were severely debarked, but only the smaller trees were not harvested (Geldenhuys 2004). Similarly, in Cameroon, Guedje *et al.* (2007) showed that most of the *Garcinia lucida* trees of 10-15 cm d.b.h. were not harvested. Nevertheless, when a species such as *Prunus africana* is highly exploited for its bark, debarked trees of all sizes were found in Cameroon (Cunningham &

Mbenkum 1993), in Madagascar (Stewart 2003) and on the island of Bioko (Sunderland & Tako 1999).

Our study highlights that the larger the debarked surface area (I6-75% of trunk debarked) the higher the amount of bark produced per year. The contrary is also true. Overall, across five species, if a tree was debarked on both sides of the trunk (I2–2 x 10% of trunk debarked and I4-2 x 20% of trunk debarked), the bark regeneration was disadvantaged. Concerning *K. senegalensis*, *L. kerstingii* and *P. biglobosa,* the higher the tree stress (i.e. following a 75% debarked trunk), the more new tissues were produced. This could be explained by a higher hormonal activity stimulated by stress in order to restore water conductivity and thus to close the wound as soon as possible. The most probable hormones to be released are auxins and cytokinines, both being involved in cell division and shoot formation (Mohr & Schopfer 1995). Moreover their highly-synergistic effect affects most of the growth processes of plants. In contrast, for *M. indica* and *P. kotschyi*, whatever the intensity of stress (20% or 75% debarked trunk), the trees' hormonal response in term of new tissue production is the same. Our experiment is not in concordance with previous observations following bark harvesting by local populations. Indeed, this percentage of 75% of trunk debarked was higher than what local people harvested on *K. senegalensis* in the same region in Central Benin. Indeed, in most cases they debarked less than 25% of the trunk and most of the trees harvested for more than 50% of their trunk bark were found near villages (Gaoue & Ticktin 2007). A similar resource inventory carried out in southern KwaZulu-Natal forests showed that on average, 43% of the total bark on the main stem per tree of *Curtisia dentata*, 31% of *Ocotea bullata* and 24% of *Rapanea melanophloeos* was removed (Geldenhuys 2004).

IMPLICATION FOR MANAGEMENT

Managers need to take objective decisions on the most appropriate harvest options for a particular species to ensure that bark harvesting is sustainable and to optimize socio-economic benefits from the resources used (Vermeulen 2009). Consequently, to provide relevant management recommendations we used our results from the two-year experiment and based on their responses to bark stripping according to season, d.b.h. and intensities of debarking (Table 3.3). In that way, it appears clearly that *M. indica* and *P. kotschyi* do not need particular recommendations on the season, d.b.h. and intensity of debarking because their response in term of edge growth is the same whatever the changes in these parameters. Thus for these species, the important aspect is the necessary delay to close the wound. To expect a good bark recovery rate for *P. biglobosa*, smaller trees (10-20 cm d.b.h.) have to be harvested during the rainy season with a debarking of 50% of the trunk. In the case of *K. senegalensis* and *L. kerstingii*, we suggest to harvested bigger trees (> 30cm d.b.h.) during the rainy season with a debarking of 75% of the circumference for *K. senegalensis* and 50% of the circumference for *L. kerstingii*. Selection of trees exhibiting rapid wound closure would therefore be a desirable practice (Neely 1988).

Acknowledgements

PART 2

ANATOMICAL FEATURES UNDERLYING THE REACTION TO BARK HARVESTING

CHAPTER 4: WOUND REACTION AFTER BARK HARVESTING: MICROSCOPIC AND MACROSCOPIC PHENOMENA IN 10 MEDICINAL TREE SPECIES, BENIN

CHAPTER 5: SIZE OF CONDUCTING PHLOEM: THE "KEY" FACTOR FOR BARK RECOVERY OF 12 TROPICAL MEDICINAL TREE SPECIES, BENIN

As the poet said, 'Only God can make a tree,' probably because it's so hard to figure out how to get the bark on.

Woody Allen

CHAPTER 4

Wound reaction after bark harvesting: microscopic and macroscopic phenomena in 10 medicinal tree species, Benin

Submitted to Trees - Structure and Function

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Summary

1. Introduction. In Africa little is known about how 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 if the ability to close the wound after bark harvesting is driven by wood anatomical changes.

2. Methods. We harvested bark from 10 medicinal tree species. 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 and density) were measured on three locations in the radial direction (before and after wounding) and on three locations around the disc surface. In addition, correlation was calculated between tissue production in closing wounds (%) and restoration of the specific conductive area (i.e. the sum of vessel area per area unit).

3. Main results. We found that during the early wound healing, all species produced vessels with smaller area than in unaffected wood and this significantly decreased the specific conductive area in eight of the investigated species. However, after two years, only six species had restored their specific conductive area. The positive correlation between the ability to produce tissue to close the wound and the ability to recover the initial conductive area two years after wounding was found with five species.

4. Conclusion. Vessels appeared to be very good anatomical indicators of the tree's reactions to debarking. Spatial changes in the wood features after bark harvesting damage were much less important than temporal changes. Recovery of the vessel features (size, density) towards their condition before wounding is a slow process that requires at least two years to complete. On the other hand, at 2 cm from the bark harvesting wound the wood was not affected.

Key-words: Africa, bark harvesting, medicinal tree species, re-growth dynamic, vessel, wound.

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 on 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 & Williams 2006). Since 2003, several bark recovery studies have been carried out such as in Zambia and Malawi (Geldenhuys & Williams 2006; Syampungani 2006; Geldenhuys et al. 2007), South Africa (Geldenhuys 2004; Vermeulen & 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 having been 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 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 & Mbenkum 1993; Vermeulen & 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* and *Albizia adianthifolia* (Syampungani 2006; Vermeulen 2006; Geldenhuys et al. 2007) and *Khaya senegalensis* and *Lannea kerstingii* (Delvaux et al. 2009) showed an effective recovery from the edges of the wound. On the other hand, 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), and *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 on 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 for protection against disturbance of their water balance whereas they should 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 & 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 & 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 two-year survey would be the minimum period required to gain reliable information on a wounded tree's survival and to analyze the mechanisms of changes in vessel features following wounding. So far, vascular system response to mechanical injury has primarily and only been studied for species from temperate regions (e.g. Li et al. 1982; Rademacher et al. 1984; Shigo 1986; Li & Cui 1988; Schmitt & Liese 1990, 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 & Aloni 1993; Thomas et al. 1995; Christensen-Dalsgaard et al. 2007). Among the twenty

tree species already studied, only one is a medicinal tree, i.e. *Eucommia ulmoides* (Li *et al.* 1982; Li & 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 would thus influence re-growth. Previous research projects focused either on relatively small harvested surface areas of bark, sometimes only a few cm² (Rademacher *et al.* 1984; Lev-Yadun & Aloni 1992; Stobbe *et al.* 2002; Frankenstein *et al.* 2005), or on total girdling, whereby all bark is removed from the trunk over a height of one to two meters (Li *et al.* 1982; Li & Cui 1988). This contrasts with this study, where the harvested proportion of bark part represented 20% or 50% of the circumference of the tree in order to mimic traditional healer practices in Benin.

The aim of this paper was to study the micro-reactions to wounding in ten medicinal tree species from Benin known to differ widely in their potential bark recovery. Vessel features can be considered as indicators of anatomical wood reactions following bark harvesting. Indeed, anatomical features have the advantage that the developmental mechanisms in response to environmental changes are permanently "archived" and can be evaluated retrospectively (e.g. Sass & Eckstein 1995). Given the important role vessels play in tree physiology, we felt it was essential to calculate the temporal and spatial impact of bark harvesting on both vessel density and area, and total conducting area. First, we investigated the impact of the wound in the course of two years both on the wound and around the trunk. Second, we hypothesized that the ability to close the wound after bark harvesting is driven by wood anatomical changes, in particular changes in specific conductive area, which represent the sum of vessel areas per unit area. To test these hypotheses, (i) features of vessels produced before bark harvesting were compared with features of vessels produced after bark harvesting; and (ii) the ability to return to the normal conductive area over a two-year period, expressed in percentage of conductive area recovery, was related to percentage of wound area that closed over the same period.

Materials and Methods

STUDY AREA

The study was done in the forest reserve the so called in French Forêt Classée des Monts Kouffé (8°30' - 8°52' N, 1°40' - 2°27' E), in Benin, West Africa. This forest of 180 300 ha belongs to the Sudano-Guinean phytogeographic region (Adomou *et al.* 2007). The average monthly temperature is from 21.0° - 33.2°C and the average annual rainfall is 1190.7mm. The study was carried out in a *Isoberlinia* spp woodland.

STUDY SPECIES AND SAMPLE COLLECTION

As a first step, a large number of medicinal tree species was selected in order to be able to compare a sufficient diversity of responses to bark harvesting. Several ethnobotanical interviews were held with traditional healers and local populations in order to learn their preference for tree species used for health care (Bockx 2004). Subsequently 10 of the most frequently used species were chosen for the study (Table 4.1). In November 2004, bark was removed from the sample trees. Wounds were made at 1 m above ground level. The wound consisted of a rectangular piece of bark 30 cm vertically and the lateral extent of the wound varied between 9 cm and 22.7 cm width, i.e. width as percentage of trunk circumference (Table 4.1). In November 2006, trees were cut at wound level and a wood disk was collected from the middle of the wound. After sampling, disks were immediately stored in FAA (formaldehyde – acetic acid – ethanol). Wood disk samples were deposited at the wood

collection of the Royal Museum for Central Africa, Tervuren, Belgium (for accession numbers: see Table 4.1).

Table 4.1: Medicinal tree species with their respective accession number Tervuren Wood (TW-sample stem disk), 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

SAMPLE PREPARATION AND WOOD ANATOMICAL MEASUREMENTS

Disk samples were embedded in PEG 2000 (PolyEthylene Glycol 2000) to keep bark and wood together. Then they were sanded using a series of sandpapers with grain size varying from 50 to 1200. On the cross-section of the disks, vessel features were measured along 8 radii (Fig. 4.1). These radii were chosen on specific locations: i) 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 starts to close the wound (W); ii) one radius on both the left and the right sides of the wound and at 2 cm from the wound (F); and iii) two radii at the opposite side of the wound on the disk sample (O). Along each radius, 11 quadrates each with an area of 3 mm² (rectangle of 2000 µm on the tangential direction and 1500 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 two-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 mm²) and vessel area (lumen area in µm²) were measured manually making use of digital image analysis software AnalySIS 3.2 (Soft Imaging System GmbH, Münster, Germany), at an optical magnification of 10. Specific conductive area represents the percentage of cross sectional area occupied by vessels per unit xylem area and was calculated for each quadrate.

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 thanks to 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)
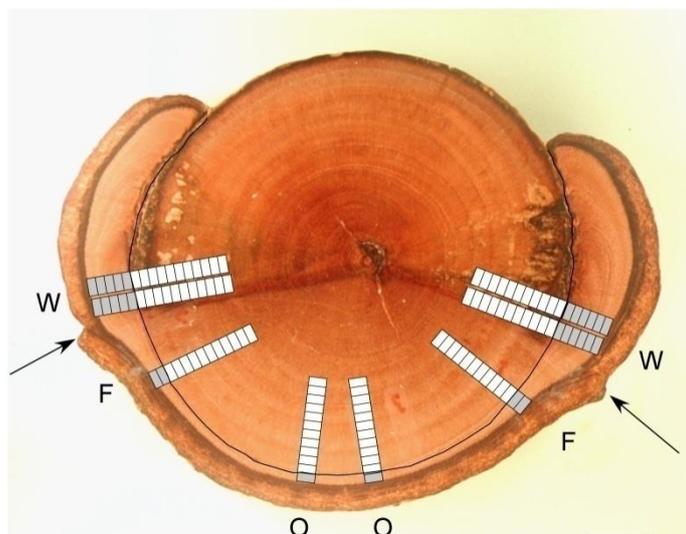
Fig. 4.1 Wood anatomical measurements were made along eight radii on the disk sample. Four radii on the wound: **W**; two radii at 2 cm from the wound: **F**; and two radii at the opposite side of the wound: **O**. The eight radial strips comprised 11 quadrates in intact wood before bark harvesting (white) and various numbers of quadrates after wounding (grey). The number of quadrates is linked to the diameter growth after a two-year period following harvesting and thus, depends on each species. The dark line draws the position of the 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

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 accomplished only on the place of wounding (W). Thus data from the four radii on the wound were gathered to measure: i) 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 ii) wound impact after a period of two years, which was done by comparing the 11 quadrates in intact wood produced before bark harvesting to the last quadrate grown two years after wounding (A2). The null hypothesis tested was that there was no difference between vessel features before and after wounding. We tested for 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 disk (at **W**ound level, 2 cm **F**rom the wound, and at the **O**pposite side of the wound) at three different moments 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 two 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 $\left(\frac{Conductive\ area\ after\ wounding}{Conductive\ area\ before\ wounding} \times 100\right)$ and the percentage of wound closure after a two-year period, a Pearson-correlation coefficient (Statistica 6.0) was calculated for the ten samples exclusively on the wound (W).

Results

VARIATION BEFORE AND DURING THE EARLY STAGE OF WOUND HEALING

The repeated measures analysis GLM showed 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. 4.2a). However, this change in vessel density was significantly higher only for *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 10 species studied: vessels became significantly smaller (Fig. 4.2b). 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. 4.2c). 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 characterise each species (Table 4.2). During the early stage of wound healing, *K. senegalensis* developped callus without vessel production (Fig. 4.2). Moreover, it was noticed that as a first reaction after wounding, *D. microcarpum* developed traumatic canals which were exceptionally large, very close to each other and observed to occur on the whole trunk circumference (Fig. 4.3). With *M. polyandra*, tyloses induced by bark harvesting were observed in vessels near the wound limit (Fig. 4.4).

DIMINISHING EFFECT OF WOUNDING WITH TIME

Over a two-year period following debarking, a significant trend towards normal vessel density was observed for *A. africana*, *B. africana*, *D. microcarpum*, *K. senegalensis*, *L. lanceolata*, *P. kotschyi* and *P. erinaceus* (Fig. 4.2a). Only four species (*D. microcarpum*, *K. senegalensis*, *L. lanceolata* and *P. erinaceus*) produced vessels with the same area as before wounding (Fig. 4.2b). In a similar way as vessel density, specific conductive area returned to normal for *D. microcarpum*, *K. senegalensis*, *L. lanceolata*, *M. indica*, *M. polyandra* and *P. erinaceus* (Fig. 4.2c). The four other species kept a significantly lower conductive area than before wounding.

SPATIAL DIMINISHING EFFECT OF WOUNDING ON CONDUCTIVE AREA

Using a two-way ANOVA (Fig. 4.5), we found that the specific conductive area measured after bark harvesting was disturbed at various distances from the wound and sometimes until two years after bark harvesting. However, wood anatomy of *M. indica* and *P. erinaceus* changed very little in time and space. A significantly lowering of the conductive area was observed only as a first reaction up 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 evidenced to still observe locally for *A. africana*, *B. africana*, *P. kotschyi* and *U. togoensis*. At two cm away from the wound, the specific conductive area of these species was not affected anymore. The conductive area of *L. lanceolata* was significantly lower up to distances of 2 cm from the wound and this situation remained during the two years of observation. Just after wounding and two years later, at the place where the wound was inflicted and at the opposite side on the disk, *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 at the opposite side of the stem disk. Indeed, at this location, its specific conductive area was significantly higher than before bark harvesting while on the wound this latter was significantly lower. Over a two-year period following harvesting, the effect of the wound on this species' reaction diminished and the difference was not significant anymore.
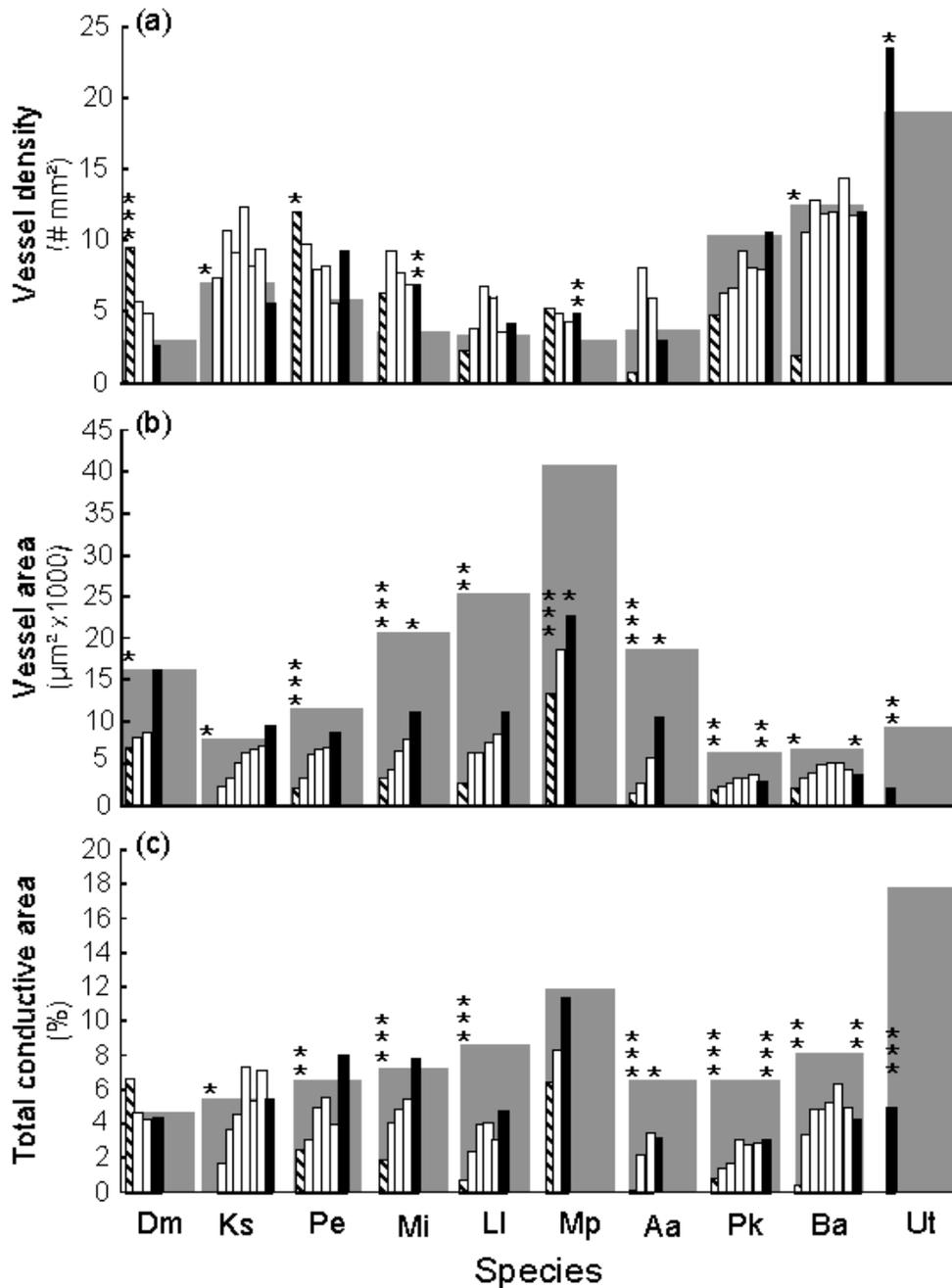
Fig. 4.2 Repeated measures GLM's of vessel density (#/mm²) (a), vessel area (μm²) (b) and conductive area (%) (c), used to test the impact of bark harvesting on vessel features before and after wounding. The different bars represent the consecutive wood-quadrates (3 mm²) produced after wounding. The broad grey bar represents the averaged value of the 11 quadrates for each of the four radii in the intact wood before bark harvesting.The hatched 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 growth the more bars. The black bar represents the last quadrate produced two 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 grey 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*.

Table 4.2: 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 (#/mm²)		Vessel area (µm²)	
	Mean ± s.d.	range	Mean ± s.d.	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	1455-62 747
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

RELATIONSHIP BETWEEN MICRO AND MACRO LEVELS

Macroscopically measured, a specie's wound closure was significantly correlated with the ability to return to its normal conductive area over a two year period after bark harvesting (Pearson-correlation coefficient $r = 0.64$, $P < 0.005$). Following this test, two groups clearly appeared (Fig. 4.6). 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 to 77.1%) but also had a good recovery of conductive area (75 to 113%) (see Fig. 4.7 for *K. senegalensis* and *M. polyandra*). *D. microcarpum* (113%) was the only species that actually showed a higher specific conductive area than what was present 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 to 21.9%) and weak recovery of conductive area (37 to 60 (74) %) (see Fig. 4.7 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 only showed some re-growth in radial direction and not tangentially.
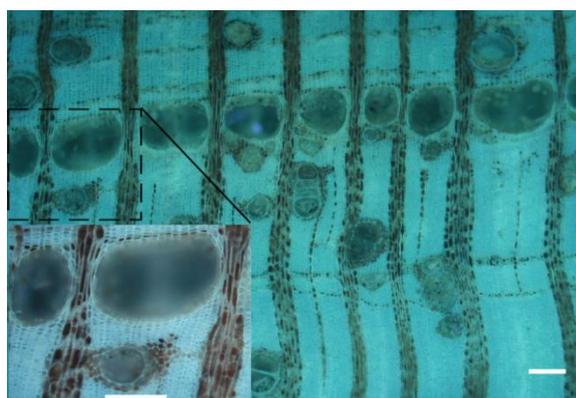
Fig. 4.3 Fluorescence microscopy of traumatic canals in secondary xylem of *D. microcarpum* on transverse section of stem disk. Formation of canals was induced by bark harvesting on the whole trunk circumference. Scale bar: 200 µm.
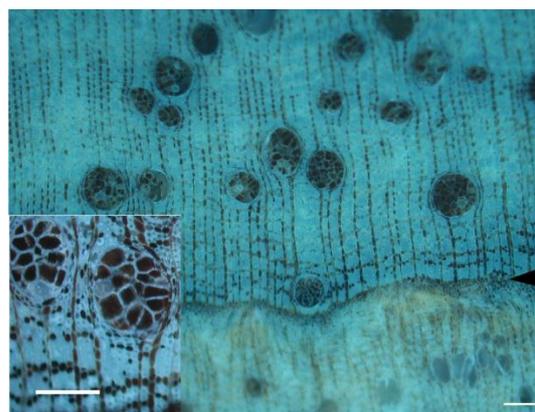
Fig. 4.4 Fluorescence microscopy of tyloses in *M. polyandra*. Vessels completely filled with tyloses on transverse section of stem disk. White arrow: position of cambium at the time of wounding. Scale bar: 200 µm.
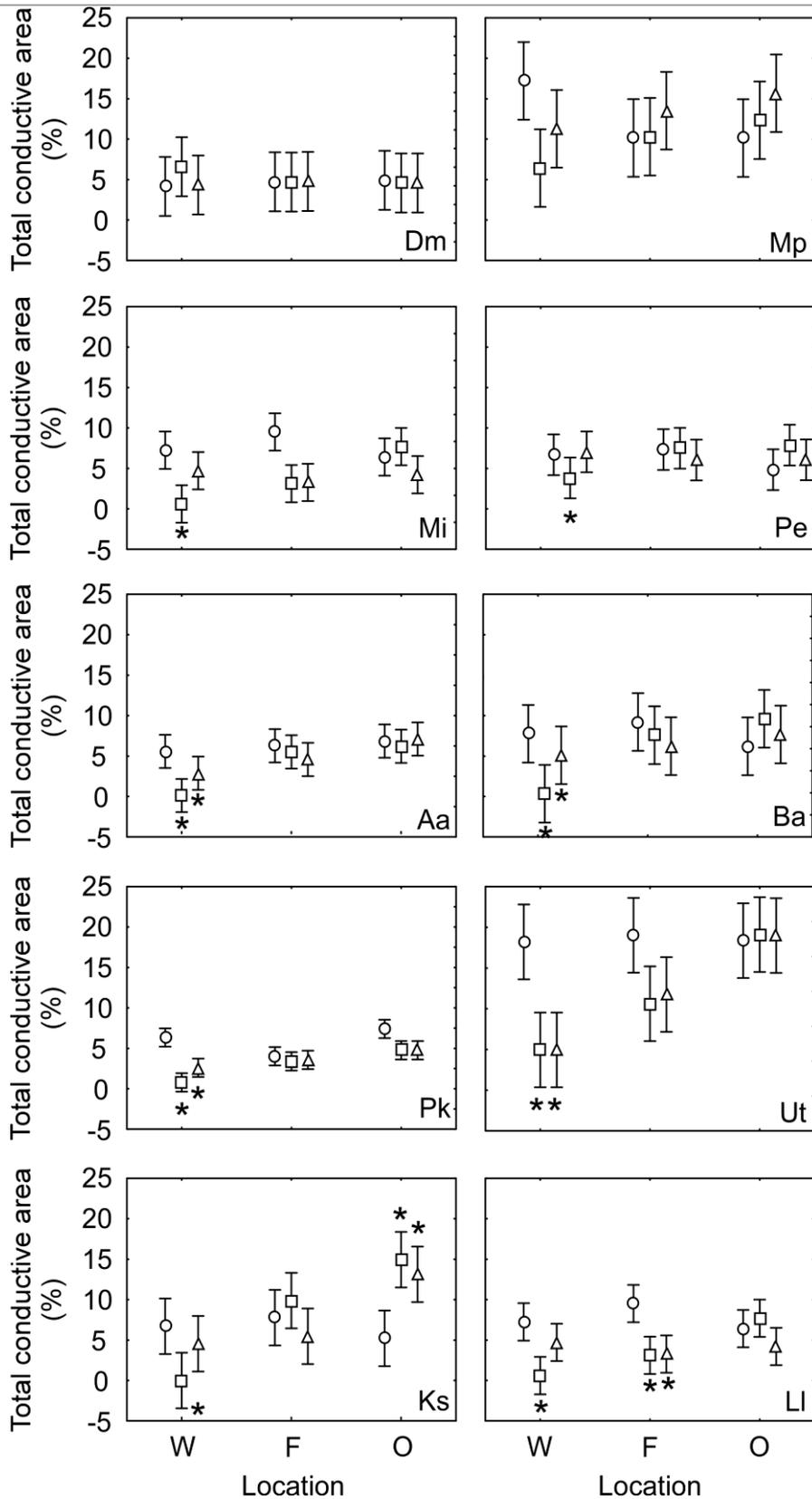
Fig. 4.5 Two-way ANOVA of conductive area from 10 medicinal tree species were used to test the impact of wounding at three different positions on the stem disk: on the wound (W); at 2 cm from the wound (F); at the opposite side of the wound (O) at three different times: before the wound (circle), during the early stage of wound healing (square) and two years after bark harvesting (triangle). *= $P < 0.05$. See Fig. 4.2 for abbreviations of species.
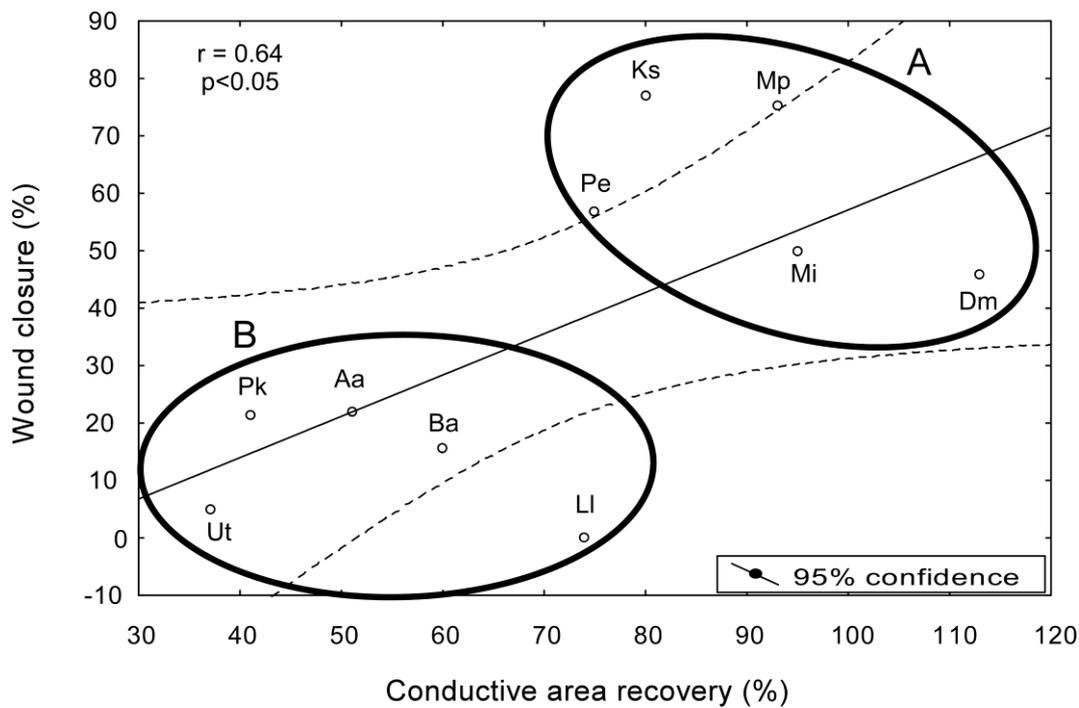
Fig. 4.6 Correlation (Pearson coefficient, r = 0.64, *P* < 0.05) between percentage of wound closure and percentage of recovery of conductive area. Two groups appear: group A where species present a good percentage of wound closure (> 40%) and of recovery of conductive area (> 70%) and group B where species display a weak percentage of wound closure (< 30%) and of recovery of conductive area (< 60%, except *L. lanceolata*). See Fig. 4.2 for abbreviations of species.

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* and *Fagus grandifolia* (Rademacher *et al.* 1984), *Acer rubrum* (Aloni & Zimmermann 1984), *Melia azedarach* (Lev-Yadun & Aloni 1993), *Betula pendula* (Novitskaya 1998), *Tilia* spp. (Stobbe *et al.* 2002), and *Populus tremula* x *Populus tremuloides* (Frankenstein *et al.* 2005; Frankenstein & Schmitt 2006). In our study, five out of the ten species showed a tendency towards increasing vessel density immediately after wounding. Nevertheless, since the vessels that were formed after injury, also were 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 & Zimmermann 1984; Shigo 1984a; Verheyden *et al.* 2005). Bark harvesting results in an obstruction to auxin flow which leads to localised auxin accumulation (Aloni & Zimmermann 1984), inducing an increase in the rate of vessel differentiation, thus resulting in more numerous but narrower vessels (Aloni & Zimmermann 1984; Aloni 1992; Mwange *et al.* 2003; Evert 2006; Frankenstein & 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 when a tree starts to protect itself after bark

harvesting is to produce 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 parenchymatic tissue without vessels, fibres 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. 4.2). Frankenstein *et al.* (2005) described that initially the formation of large, thin-walled parenchymatous cells appeared along the wound edge and thus two different strategies of callus formation would be developed during the first 70 days after wounding in *Populus tremula* x *Populus tremuloides*: (i) formation of a wound cambium within the parenchymatous zone as a tangential extension of the undisturbed cambium, or (ii) 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 included phloem, we may assume that our ten species follow the first strategy to close the wound from the edge.
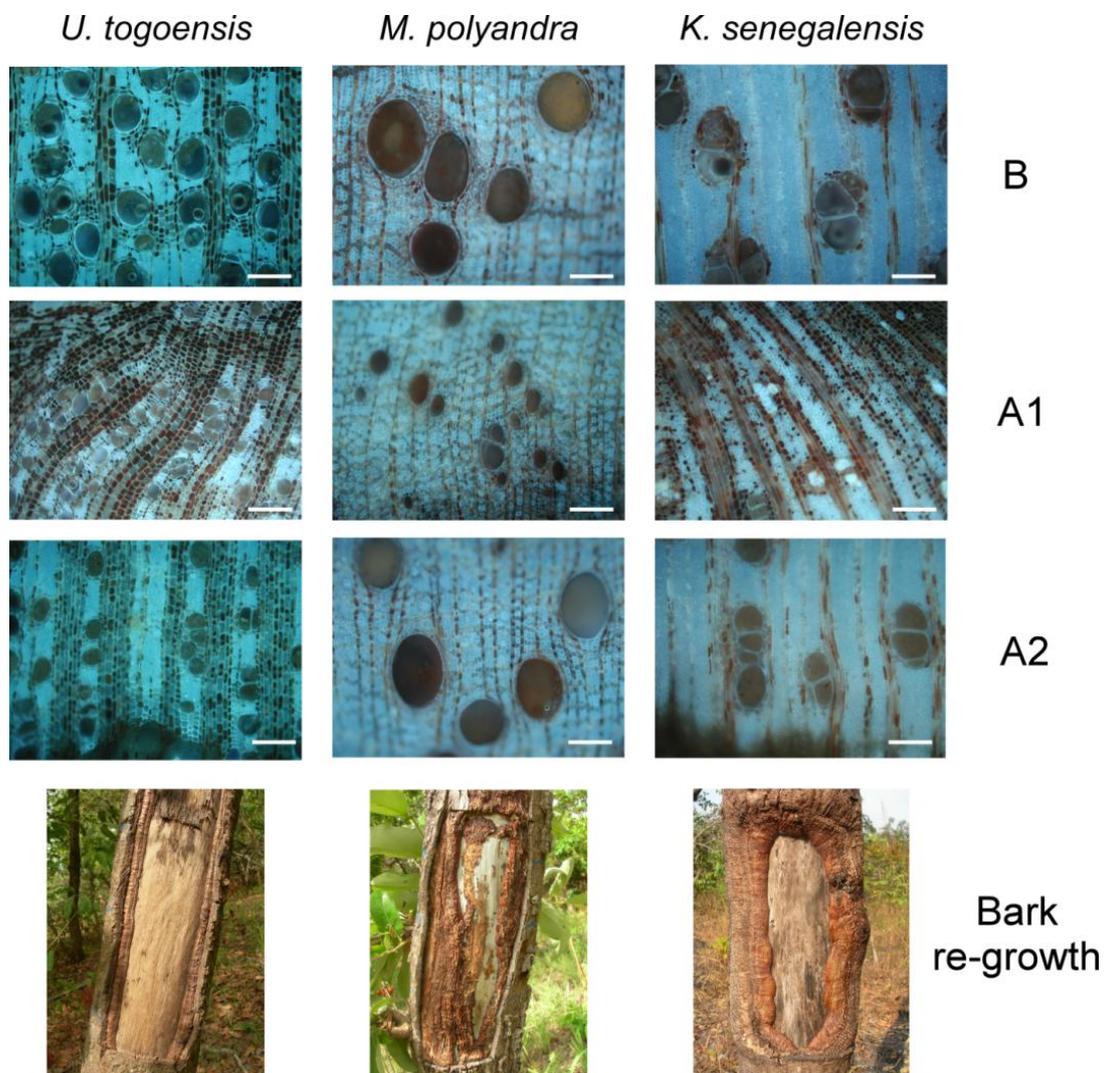
Fig. 4.7 Fluorescence microscopy of vessel features (density and size) on transverse section of stem disks and macroscopic view of bark regeneration on tree stems in the Forêt Classée des Monts Kouffé (Benin). Vessels before bark harvesting (B), at early stage of wood healing (A1) and after a period of two years (A2), and bark: edge growth for *U. togoensis* and *K. senegalensis* and sheet growth for *M. polyandra*. Scale bar: 200 µm.

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 defence 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 spread of pathogens throughout the plant via the plugging of xylem vessels (Clerivet *et al.* 2000).

DIMINISHING BARK HARVESTING IMPACT WITH TIME

With *D. microcarpum*, *K. senegalensis*, *L. lanceolata* and *P. erinaceus*, the impact of wounding had completely disappeared within two years following bark harvesting: as indeed both vessel area and density, and conductive area had 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* had formed normal-sized vessels two years after wounding (Rademacher *et al.* 1984; Lev-Yadun & Aloni 1993). Frankenstein and Schmitt (2006) also observed for *Populus tremula* X *P. tremuloides* that modifications in lignin distribution in newly formed xylem elements at wound edges occurred less frequently and finally completely disappeared over a two-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. Thus we have to pay attention. Even if the specific conductive area has regained the level than before the wounding, the hydraylic conductivity has not necessarily (Fig. 4.8). This is link to the fact that hydraulic conductivity is proportional to the fourth power of radius and consequently the small vessels did not contribute so much to conductivity than big ones. 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 two years after debarking. Thus, for these species 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.
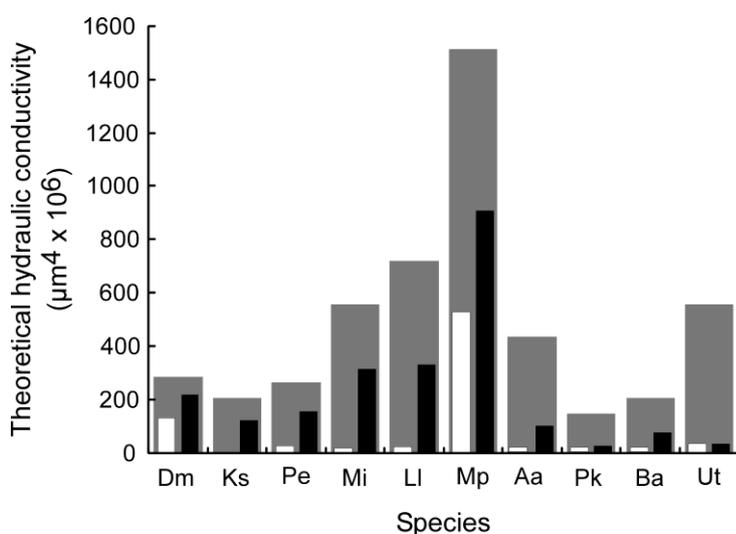
Fig. 4.8 Simulation of theoretical hydraulic conductivity of the xylem based on the Hagen-Poiseuille equation. Vessel was considered as a circle and the radius was calculated from the measured area of vessel. See Fig. 4.2 for abbreviations of species. The broad grey bar represents the averaged value 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 two years after wounding.

SPATIAL DIMINISHING WOUND EFFECT ON CONDUCTIVE AREA

In eight of our ten studied species, 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 disk opposite the wound.

This interesting finding implies 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. Despite in some cases where a large part of the trunk was wounded (suggesting interruption of water transport), we never observed 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 & Zimmermann 2002; Kitin *et al.* 2004; Loepfe *et al.* 2007). They deducted that the xylem is 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 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 further spread in a longitudinal direction than in a transversal direction.

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 species (Fig. 4.6). In group B, the absence of regaining a normal-looking conductive area two years after bark harvesting was explained by vessel size which stayed significantly smaller than before the wound was applied (Fig. 4.2b). 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 are located in the same area, inside Forêt Classée des Mont Kouffé, hence they grew under similar climate. After wounding, vessel formation was to be mainly considered as controlled by internal factors. As auxin is mainly transported through mature phloem, any damages to the bark result 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) showed that auxin content decreases at advanced stages of bark recovery and, that as a consequence, lower auxin concentration will induce slower differentiation and therefore initiate fewer and larger vessels (Aloni & Zimmermann 1984; Aloni 1987, 1992). In view of our results, *A. africana*, *B. africana*, *P. kotschyi* and *U. togoensis*, species belonging to group B (weak wound closure), should show altered auxin-production even two 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 to compartmentalize the wounded stem part to avoid the spread of insect

attacks and fungi within the trunk, rather than to form 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 two-year period.

Conclusion and perspectives

In conclusion, our results provide new insights into the spatial changes in the wood after bark harvesting were much less important than temporal changes. Recovery of the vessel features towards the condition before wounding is a slow process that requires at least two years to complete. While at 2 cm away from the bark harvesting wound, the specific conductive area was usually not affected. Moreover, in relation to previous studies (e.g. Loepfe *et al.* 2007) we propose that after that kind of bark harvesting, trees may continue their normal sap flow for photosynthetic activity through their network of interconnected xylem vessels. Trees which 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.

Acknowledgements

CHAPTER 5

Size of conducting phloem: the "key" factor for bark recovery of 12 tropical medicinal tree species

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Summary

1. Introduction. In Africa, bark is a popular source of medicines for rural populations. Despite the importance of bark, no anatomical studies were carried out to understand the variety of responses following bark harvesting observed in medicinal tree species all over Africa. The present study aims to determine the anatomical variable(s) that could help predicting the potential of 12 medicinal tree species to show a good bark recovery rate.
2. Methods. Discs of branches were collected from non-harvested trees of the 12 species. A total of 12 anatomical variables were measured in wood, cambium and phloem zone and the correlation between the bark recovery rate and each variable was tested.
3. Main results. Among the 12 anatomical variables tested, the thickness of the conducting phloem zone emerged as the most important one to explain the bark recovery rate. The presence of sclereids within the conducting phloem zone was also an explanatory variable, in negative correlation with the bark recovery rate. For 10 out of the 12 species, the thickness of the cambial zone varied significantly with season, nevertheless this variable did not contribute significantly to the explanation of the bark recovery rate.
4. Conclusion. Given that the 12 studied species showed a large range of bark recovery rates (0.1 to 10.0 cm/y), we assume that they are representative of the variety of wound healing responses (i.e. wood and bark tissue production) in a high number of African tree species. Consequently, our results offer the advantage to foresee the potential of wound closure in any tree from which bark could be harvested.

Key-word: bark regeneration, cambium, phloem, prediction, sclereids, West Africa, wood.

Introduction

Bark, all tissues outside the vascular cambium (Evert 2006), is the protective barrier of the tree against external attacks and dessication and allows the transport of photosynthates from leaves to roots. Bark tissue is not only indispensable for the life of the tree itself. All over Africa, bark is abundantly harvested for its medicinal properties (Cunningham 1993; Grace *et al.* 2002; Guedje *et al.* 2007). It forms one of the ingredients of among others maceration (method of extraction by soaking parts of plants in water) and decoction (method of extraction by boiling of plant material which may include stems, roots, bark and rhizome) used in primary health care. In South Africa, bark is the most popular medicinal product harvested from trees and accounts for 31% of the plant tissue harvested and traded annually in KwaZulu-Natal (Geldenhuys & Williams 2006). In Benin, a nationwide ethnobotanical survey (Adjanohoun *et al.* 1989) showed that bark represents 10.5% of the medicinal plant products, and that 31.5% of the country's tree species are used for their bark. However, it is well known that harvesting bark can be highly damaging in terms of tree survival (Cunningham 1991; Peters 1994; Geldenhuys 2004; Vermeulen 2006) and that uncontrolled commercial harvesting of medicinal plants may soon lead to resource depletion. That is explained by the fact that besides the harvesting intensity, a species' vulnerability to bark harvesting depends on its capacity to recover from bark stripping (Geldenhuys & Williams 2006). Indeed, bark regeneration involves the replacement of harvested tissues at the place of wounding and this restoration follows a specific developmental pattern: formation of a callus, a ligno-suberised layer, a wound cambium, a wound xylem and wound phloem (e.g. Biggs 1992; Oven & Torelli 1999; Stobbe *et al.* 2002; Frankenstein *et al.* 2005).

In view of the ethnobotanical and economic importance of bark tissue, it was a surprise to notice that so far no anatomical studies were carried out to clarify the different regeneration capacities of the tropical tree species, known worldwide for the medicinal use of their bark (*Prunus africana*, *Alstonia constricta*, *Cinchona officinalis*, etc.). The studies that have been done focused on either bark recovery or anatomy but not on both in combination. A few studies were carried out under natural conditions to follow the bark recovery of some African tree species after an experimental bark harvesting (Vermeulen 2006; Geldenhuys *et al.* 2007; Guedje *et al.* 2007; Delvaux *et al.* 2009), or evaluate the impact on tree survival after uncontrolled bark harvesting by stakeholders (e.g. Cunningham & Mbenkum 1993; Geldenhuys 2004). Previous investigations on the anatomical and hormonal changes during the early stages of the bark healing process (e.g. Li & Cui 1988; Aloni 1992; Lev-Yadun & Aloni 1992; Schmitt & Liese 1993; Thomas *et al.* 1995; Oven & Torelli 1999; Cui & Li 2000; Grünwald *et al.* 2002; Frankenstein *et al.* 2005; Pang *et al.* 2008), focused especially on temperate tree species. For these earlier studies it is concluded that there are two ways of wound healing via (i) surface callus (*Eucommia ulmoides*, *Tilia* sp.) (Li & Cui 1988; Stobbe *et al.* 2002) or (ii) callus at the wound edge (e.g. *Populus tremula* x *Populus tremuloides*, *Betula pendula*, *Fagus sylvatica*, *Quercus robur*, *Albies alba*, *Picea abies*, *Pinus sylvestris*, *Larix decidua*) (e.g. Grünwald *et al.* 2002; Frankenstein *et al.* 2005). Besides, it is important to distinguish which tissues (axial parenchyma phloem, cambium, immature xylem, ray parenchyma) participate specifically and preferentially in the early stage of wound healing.

Recently (see Chapter 3), Delvaux *et al.* found a wide range of bark recovery (0.1 to 10.0 cm/y) when studying 12 African medicinal tree species for their reaction after bark harvesting. Given the importance of bark harvesting in a large number of African tree species and the variable capacity to recover, we decided to explore the relationship between the bark recovery rates of different tree species and their anatomical characteristics of wood and bark.

The present study investigated the potential of several anatomical variables to predict the re-growth capacity after bark harvesting of 12 tree species in Benin. More specifically, the

objectives of the study were: (i) to describe the anatomy of the zone of immediate interest for the process of wound healing i.e. the wood and the bark tissue near the cambium, (ii) to analyse the variance of bark recovery rates in relation to the potential explanatory anatomical variables.

Material and methods

STUDY AREA

The study was conducted in the "Forêt Classée des Monts Kouffé" (8°30' - 8°52' N, 1°40' - 2°27' E) which is situated in central Benin, West Africa. This area covers 180,300 ha within the Sudano-Guinean phytogeographic region (Adomou *et al.* 2007). The average monthly temperature is 21.0°–33.2°C and the average annual rainfall is 1139.1 mm (Agence pour la Sécurité de la Navigation Aérienne en Afrique et à Madagascar (ASECNA) in Benin). The study was carried out in deciduous woodland.

STUDY SPECIES AND SAMPLE COLLECTION

As a first step, a number of medicinal tree species were selected so as to compare a sufficient diversity of responses to bark harvesting. Ethnobotanical interviews were conducted with traditional healers and local people in order to learn their preferences for tree species used for health care (Bockx 2004). Subsequently, 12 of the most frequently used trees species were chosen for a study of bark recovery (Table 5.1) over a two-year period following bark harvesting (see Chapter 3). To explain the results, we concentrated on the same 12 species from the Forêt Classée des Monts Kouffé to collect samples. Entire discs of branches were collected to be sure that the cambium and the bark remained together since trunk samples split immediately in wood and bark when harvested. Sampling was done in March 2005 (dry season) and in November 2006 (rainy season) on the 12 species.

Table 5.1: The 12 tree species used in this study. The range of theoretical diameter at breast height (d.b.h.), tree height (m) and bark recovery rates (cm/year, mean ± SE) (see Chapter 3) are given.

Species	Family	Height (theoretical) (m)	dbh (theoretical) (cm)	Bark recovery rates cm²/year
Afzelia africana Sm.	Fabaceae (C)	25-30	40-60	0.3 ± 0.2
Burkea africana Hook.	Fabaceae (C)	10-12 (20)	40-60	0.4 ± 0.2
Detarium microcarpum Guill. & Perr.	Fabaceae (C)	08-10	20-30	1.5 ± 0.2
Khaya senegalensis (Desv.) A. Juss.	Meliaceae	25-35	40-70	10.0 ± 0.2
Lannea kerstingii Engl. & K. Krause	Anacardiaceae	12	40-60	9.0 ± 0.3
Lophira lanceolata Van Tiegh. ex Keay	Ochnaceae	08-10	20-30	1.2 ± 0.2
Mangifera indica L.	Anacardiaceae	10-15 (30)	20-30	4.1 ± 0.2
Maranthes polyandra (Benth.) Prance	Chrysobalanaceae	06-08	15-25	0.1 ± 0.2
Parkia biglobosa (Jacq.) R. Br. ex G. Don	Fabaceae (M)	10-15	30-50	4.0 ± 0.3
Pseudocedrela kotschyi (Schweinf.) Harms	Meliaceae	09-12	20-30	3.2 ± 0.2
Pterocarpus erinaceus Poir.	Fabaceae (P)	08-12	30-50	2.0 ± 0.2
Uapaca togoensis Pax	Euphorbiaceae	10-15	20-30	0.7 ± 0.2

(C): Caesalpinioideae; (M): Mimosoideae; (P): Papilionoideae

SAMPLE PREPARATION AND MICROSCOPIC ANALYSIS

After sampling, branches were immediately fixed in FAA (formaldehyde-acetic acid-alcohol). Each disc sample was cut into four blocks. These blocks were embedded for 72 h with PEG2000 (PolyEthylene Glycol) at 60°. Samples were sectioned in transverse sections (18-20 µm thickness) with sliding microtome (Microm). The microscopic slides were progressively dehydrated in an ethanol series and double stained with Safranin O (Merck)-Fast Green FCF (C.I. 42053, Merck). Measurements were made with the image analysis software AnalySIS Pro 3.2 (Soft Imaging System GmbH, Münster, Germany) with a microscope Olympus BX60.

ANATOMICAL MEASUREMENTS

A total of 12 anatomical variables were measured in the wood and bark zone. In the wood zone, the percentage surface area of each tissue was measured semi-automatically: vessels (VeXy, fibres (FiXy), rays (RaXy), parenchyma (PaXy) and intercellular canals (CaXy). These measurements were made using 15-20 rectangles (1000 µm on the tangential direction and 800 µm on the radial direction) immediately in contact with the cambial zone. In the bark zone, tissue proportions were measured only in the conducting phloem zone: sieve tubes were taken together with axial phloem parenchyma (CPPhl) as suggested by Trockenbrodt (1994b), fibers (FiPhl), rays (RaPhl) and intercellular canals (CaPhl). Conducting phloem was considered as phloem tissue with open sieve tubes following the definition of Lev-Yadun & Aloni (1991). Moreover, as thickness of conducting phloem was variable amongst species and samples, the size of measuring fields was also variable but only in the radial direction. The thickness (µm) of the conducting phloem (CP) and the thickness (µm) of the cambial zone (ZC) were measured as variables, as was the presence or absence of sclereids in the conducting phloem zone (SclPhl).

STATISTICAL ANALYSIS

One-way ANOVA analyses were performed to test the relationship between the wound recovery response and 12 wood anatomical variables in the 12 studied species. Firstly, simple linear regressions were used to study the relationship between the effect of the 12 anatomical variables and bark recovery rate. Second, a multiple linear regression was computed to determine the two most explanatory variables. When the Shapiro-Wilk's W test showed a non-normal distribution, a square root or arcsine transformation was performed to comply with the assumptions of an ANOVA. All analyses were carried out in STATISTICA 6.0.

Results

ANATOMICAL CHARACTERISTICS OF THE XYLEM ZONE

In *L. kerstingii*, *P. erinaceus* and *M. indica*, more than 50% of the wood surface area was occupied by fibres that was significantly different from other species (Fig. 5.1). In contrast, fibers covered a significantly limited area in *U. togoensis*, *P. kotschyi* and *L. lanceolata* (13.4%, 17.7% and 25.9%). The largest area occupied by vessels was obtained by *P. kotschyi* and *U. togoensis*. These two species were significantly different from the 10 other species (Fig. 5.1) and particularly from *A. africana*, *P. biglobosa*, *P. erinaceus* and *M. polyandra* which showed a rather low proportion of vessels. The axial xylem parenchyma was significantly more abundant in *A. africana*, *L. lanceolata* and *P. biglobosa* (35.5%, 34.5%,

and 32.5%) while it was scarce in *L. kerstingii* and *K. senegalensis* (6.3% and 10.9%). The surface area of wood rays was more or less the same within the 12 species. However, the lowest surface area of wood rays was measured in *P. erinaceus* and *P. biglobosa* and the largest surface area of wood rays was found in *K. senegalensis* and *U. togoensis*. Intercellular canals were observed in five species: *B. africana*, *D. microcarpum* (Fig. 5.2), *K. senegalensis* (so small to see), *P. biglobosa* (so small to see) and *P. kotschyi*.
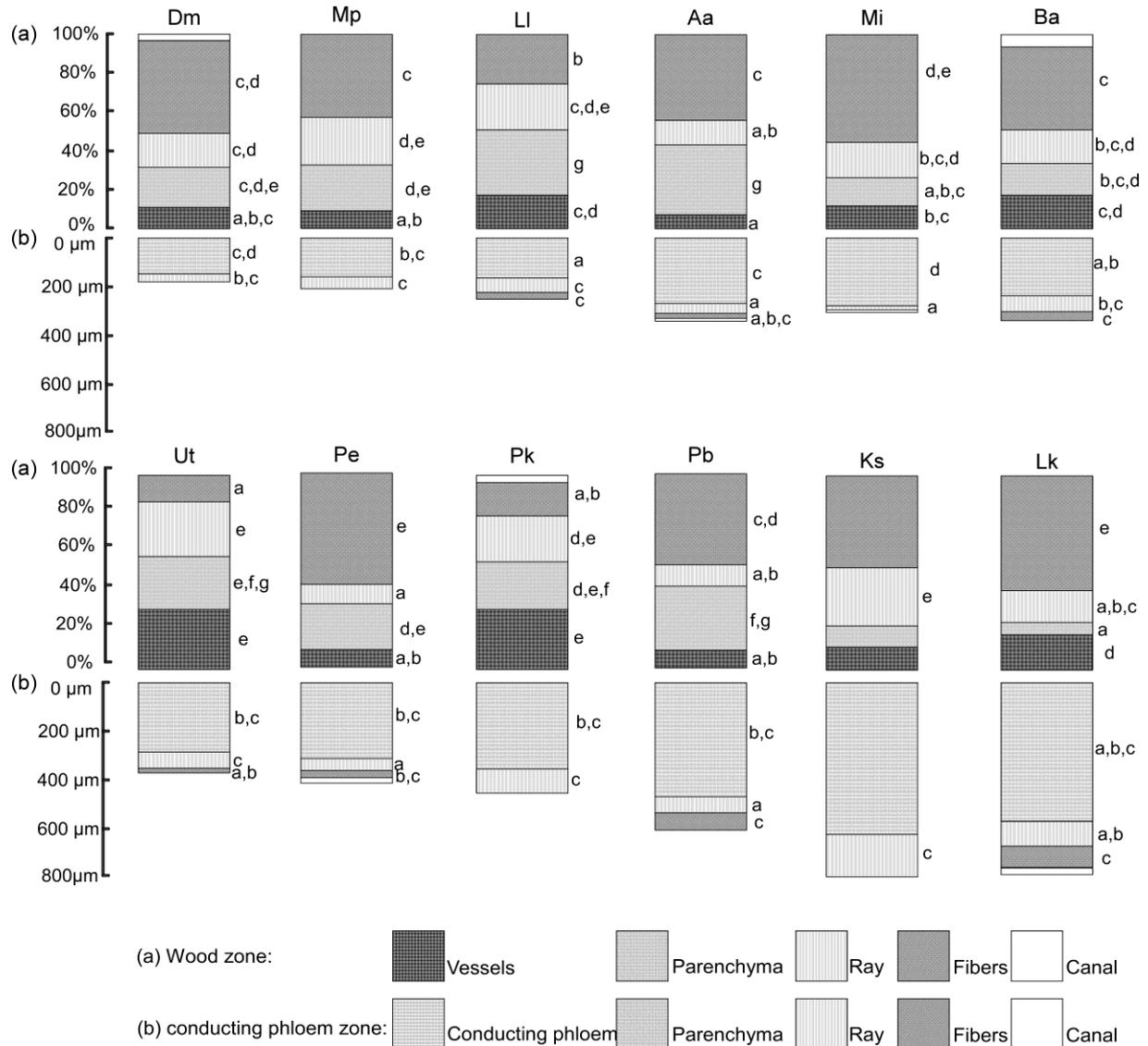
Fig. 5.1: Branch tissue proportion of (a) Wood zone vessels, parenchyma, rays, fibres and intercellular canals as seen in transverse sections, expressed as percentage of the measured area (constant); (b) Conducting phloem zone parenchyma cells + sieve tubes, rays, fibres and intercellular canals as seen in the transverse sections, expressed as percentage of the measured area (variable from species to species, in μm). Identical small letters indicate no significant difference at the $P \leq 0.05$ confidence level (ANOVA one-way). Aa: *Afzelia africana*, Ba: *Burkea africana*, Dm: *Detarium microcarpum*, Ks: *Khaya senegalensis*, Lk: *Lannea kerstingii*, Ll: *Lophira lanceolata*, Mp: *Maranthes polyandra*, Mi: *Mangifera indica*, Pb: *Parkia biglobosa*, Pe: *Pterocarpus erinaceus*, Pk: *Pseudocedrela kotschyi*, Ut: *Uapaca togoensis*.

ANATOMICAL CHARACTERISTICS OF THE CONDUCTING PHLOEM ZONE

No fibres were found in the conducting phloem zone of *D. microcarpum*, *K. senegalensis*, *M. indica*, *M. polyandra*, and *P. kotschyi*. In the other seven species, a low percentage of fibres was measured with values between 2.1% to 11.6%. As in the wood, the surface area occupied by rays was more or less similar for all species (Fig. 5.1). While, *M. indica*, *P. biglobosa*, *P. erinaceus* and *A. africana* showed the lowest percentage of phloem rays (7.1% to 11.6%), *M. polyandra* and *L. lanceolata* had the highest percentage of surface area occupied with rays (23.4%). The percentage of phloem parenchyma cells and sieve tubes in the conducting phloem was relatively similar between species and ranged from 65% (*L. lanceolata*) to 91.4% (*M. indica*). Intercellular canals were found in four species: *A. africana*, *L. kerstingii* (Fig. 5.3), *M. indica* and *P. erinaceus*.
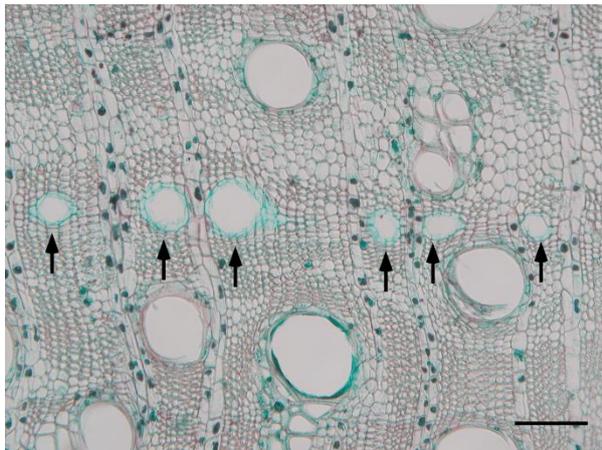
Fig. 5.2: Intercellular canals (arrows) in wood of *Detarium microcarpum*. Scale bar: 200μm
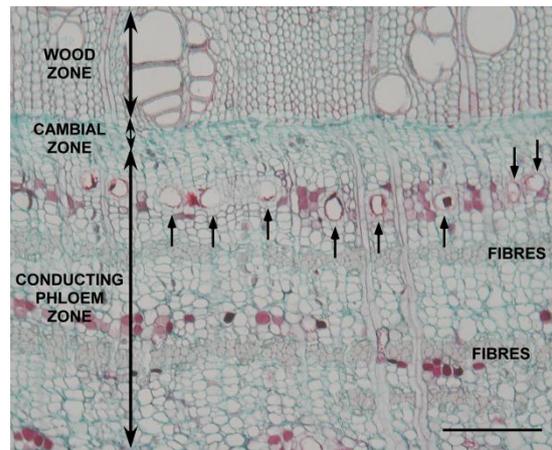
Fig. 5.3: Intercellular canals (arrows) in conducting phloem of *Lannea kerstingii*. Scale bar: 200μm

SEASONAL VARIATION OF THE CAMBIAL ZONE

For 10 out of the 12 species, the thickness of the cambial zone varied significantly with season (Fig. 5.4a). As could be expected, the cambial zone was generally smaller during the dry season (23.1 μm to 86.4 μm) than during the rainy season (53.0 μm to 124.7 μm). Whatever the season, the same size was measured for *P. biglobosa* and *P. erinaceus*. The annual average cambial zone was significantly thinner in *B. africana* and *D. microcarpum* than in *M. indica* and *P. kotschyi* (Fig. 5.4b). Nevertheless, the eight other species were no significantly different from each other (Fig. 5.4b).

INTERSPECIES DIFFERENCES IN CONDUCTING PHLOEM SIZE

The thickness of the conducting phloem varied highly amongst species with value ranging 158.2 μm to 695.2 μm (Fig. 5.1). Maximum thicknesses were observed in *P. biglobosa*, *L. kerstingii* and *K. senegalensis* and the minimum ones occured in *D. microcarpum* and *M. polyandra*. The sclereids were only found in *L. lanceolata*, *M. polyandra* and *U. togoensis* within the conducting phloem zone. For these species, the proximity of sclereids with the cambial zone was high: 16.7 μm (*L. lanceolata*), 36.0 μm (*U. togoensis*) and 104.0 μm (*M. polyandra*).
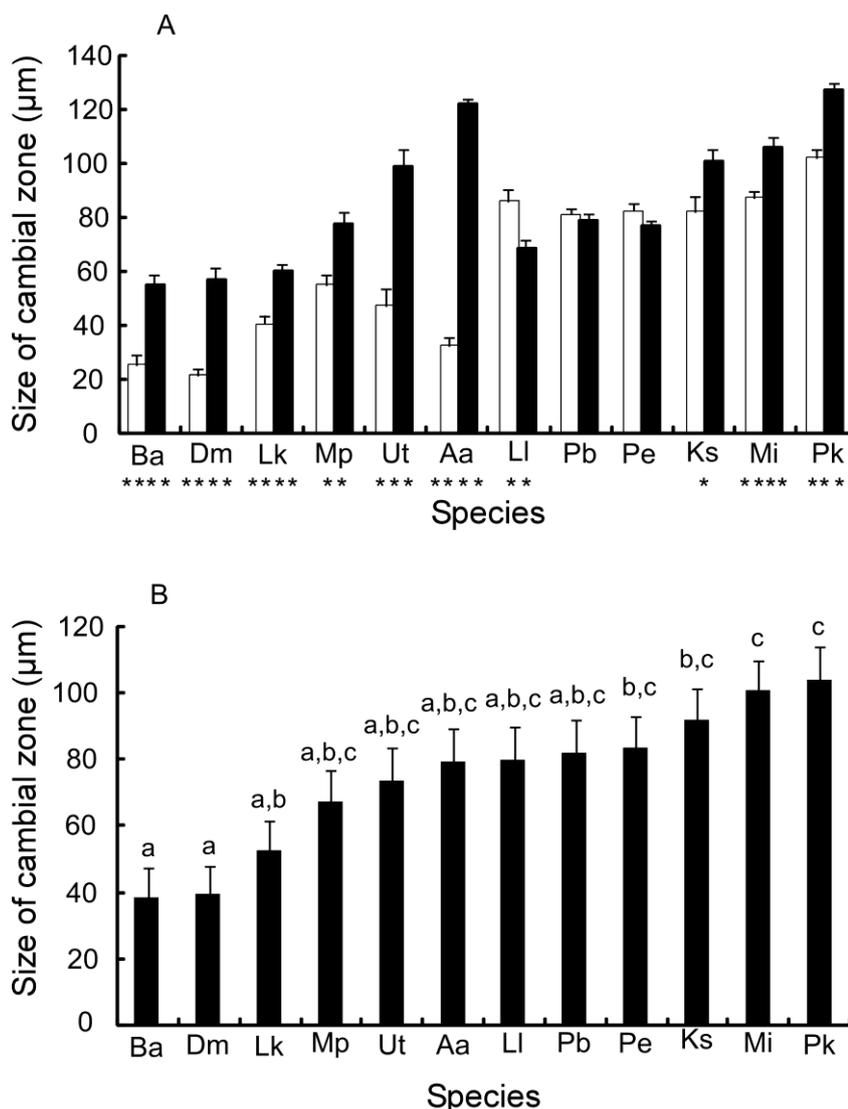
Fig. 5.4: A: Results of one-way ANOVA's, testing for differences in width of cambial zone (µm) between the dry season (light bars) and the rainy season (dark bars) for the 12 studied species. Errors bars correspond to standard errors. $*P < 0.05$, $**P < 0.01$, $*** P < 0.001$, $****P < 0.0001$. B: Results of one-way ANOVA's, testing for differences in mean annual width of cambial zone (µm) between the 12 studied species. Identical small letters indicate no significant difference at $P \leq 0.05$ confidence level. See Fig. 5.1 for abbreviations.

BARK REGENERATION AND ANATOMICAL CHARACTERS

Table 5.2 gives the results of simple linear regressions of bark regeneration against anatomical variables. Thickness of the conducting phloem zone (CP) emerged as the most important factor explaining the positive correlation with bark regeneration (53.2% of total variance). The percentage of parenchyma in the wood (PaXy) was the second best explanatory variable (26.9%), showing a negative correlation with bark recovery rate. The presence of sclereids within the conducting phloem zone was the third most explanatory variable (23.0%) with increase of bark regeneration rate when sclereids were absent of conducting phloem zone. The two next variables were singled out: percentage of fibres in the wood (FiXy) and percentage of rays in the conducting phloem zone (RaPhl). The seven other variables were not significant ($P > 0.05$).

Table 5.2: Simple linear regressions between bark recovery rates of 12 medicinal tree species and anatomical explanatory variables ($y = b_0 + b_1x$)

Explanatory variables x	Minimum	Mean	Maximum	r	r^2	P^a
Conducting phloem zone (µm)	99.490	365.328	1000.778	0.73	0.532	**< 0.001**
Xylem parenchyma (%)	1.049	22.273	53.200	-0.52	0.269	**< 0.001**
Sclereids	0.000	0.238	1.000	-0.48	0.230	**< 0.001**
Xylem fibres (%)	3.231	42.038	68.254	0.30	0.091	**0.001**
Phloem ray (%)	2.434	16.721	35.747	-0.19	0.036	**0.032**
Cambial Zone (µm)	11.593	73.443	177.500	0.15	0.021	0.104
Xylem canal (%)	0.000	0.972	14.966	-0.11	0.012	0.220
Phloem canal (%)	0.000	0.865	13.543	0.09	0.008	0.322
Xylem ray (%)	5.944	19.522	38.600	0.08	0.007	0.348
Conducting phloem (%)	49.664	77.511	96.815	-0.08	0.006	0.365
Xylem vessels (%)	0.000	15.219	38.393	0.06	0.003	0.516
Phloem fibres (%)	0.000	5.019	27.017	0.05	0.003	0.559

Variables are classified in descending order of explained variance (r^2). r values are Pearson correlation coefficients. Conducting phloem (%) = sieve tubes + phloem parenchyma.
[a]Probability that b1 = 0

As CP explains 53.2% of total variance, we carried out a multiple regression with CP and the four other significant explanatory variables (Table 5.3) in order to determine the best set of anatomical variables able to express the bark recovery rates. All different sets of variables contributed significantly to the explanation of the bark recovery rate. Nevertheless, the four combinations provided low explanatory models for individual bark recovery rates (r^2 comprised between 0.55 and 0.60). Consequently we considered that only CP was the best explanatory variable.

Table 5.3: Multiple linear regressions between bark recovery rates of 12 medicinal tree species and two anatomical explanatory variables ($y = b_0 + b_1x_1 + b_2x_2$)

x_1	x_2	r^2	b_0	b_1	b_2	P for b_1^a	P for b_2^a
CP (µm)	Wood Parenchyma (%)	0.601	-5.30	0.62	-0.280	0.000	< 0.0001
CP (µm)	Sclereids (pres/abs)	0.590	-9.08	0.64	-0.260	0.000	< 0.0001
CP (µm)	Wood Fibers (%)	0.562	-15.28	0.70	0.174	0.000	0.0048
CP (µm)	Phloem Ray (%)	0.554	-10.24	0.72	-0.150	0.000	0.0165

Models are classified in descending order of explained variance (r^2). Only models with both slopes b_1 and b_2 significantly different from 0 ($P < 0.05$) are shown. CP = conducting phloem zone.
[a]Probability that b_1 or b_2 = 0

Discussion

A fast bark recovery after harvesting seemed to depend mainly on a thick zone of conducting phloem. This result is in good agreement with previous work on temperate species studying the regeneration pattern along the edge of a wound. Frankenstein *et al.* (2005) described two strategies of cambium regeneration and callus formation in which phloem parenchyma cells play an important role in the first step toward the recovery process of bark and wood after wounding. In most cases phloem parenchyma cells that dedifferentiate to develop the callus at

the wound edge (Oven & Torelli 1994; Hawkins & Boudet 1996; Oven *et al.* 1999), whereas cambial cells and undifferentiated xylem cells are hardly involved. The formation of callus at the wound edge and the formation of a continuous ligno-suberised layer on the outer surface of the callus are essential steps towards the differentiation of a new cambium within the callus (Novitskaya 1998; Oven & Torelli 1999). However, in some cases when wounding was restricted to bark, the wound cambium dedifferentiated within the phloem tissue such as in *Salix caprea*, *Tilia tomentosa* (Trockenbrodt 1994a), *Populus tremula* x *Populus tremuloides* (Frankenstein *et al.* 2005) and in *Hevea brasiliensis* (Thomas *et al.* 1995). The link between a fast bark recovery and a thick zone of conducting phloem explains the low annual bark regeneration rate of *M. polyandra* and *L. lanceolata* compared to *L. kerstingii* and *K. senegalensis* (Table 5.1). The thickness of their conducting phloem was indeed significantly different between *M. polyandra* and *L. lanceolata* (182.6 µm and 218.7 µm) and *L. kerstingii* and *K. senegalensis* (687.4 µm and 695.2 µm) (Fig.5.1). In our study, the conducting phloem zone was mainly composed of axial parenchyma cells, associated with the sieve tubes (65.0% to 91.4%) and few phloem fibres, sclereids and phloem rays. These percentages were similar to those found by Trockenbrodt (1994b) in *Quercus robur* L. (82%), *Ulmus glabra* Huds. (82%), *Populus tremula* L. (60%) and *Betula pendula* Roth (48%).

Nevertheless, the thickness of the conducting phloem explained only 53.2% of the variance in bark recovery rate and even associated with other anatomical variables, a maximum of 60.1% of the variance could be explained. That means that additional non-anatomical variables, such as the hormonal household of the different species, play also a big role in wound healing (e.g. Benayoun *et al.* 1975; Aloni 1992; Lev-Yadun 2002; Mwange *et al.* 2003; Aloni 2004).

It could be surprising that the size of the cambial zone was not significantly correlated with the bark recovery rate although it was shown before that cambial cells pre-existing before wounding, contributed to the callus formation (Li & Cui 1988; McDougall & Blanchette 1996; Oven & Torelli 1999; Grünwald *et al.* 2002; Stobbe *et al.* 2002). However, in the species studied by these authors, bark regeneration took place via surface callus while in the present study recovery from the wound edge was the main repair process. In the case of bark regeneration from the wound edge, the contribution of cambial cells to wound healing was proven to be smaller than the contribution of the parenchyma phloem cells (Frankenstein *et al.* 2005). Next to the way of bark regeneration, from the wound surface or from the edge, also tree species determines which tissues are involved in wound closure. Depending on the species, different tissues take part, in combination or exclusively, to the callus formation. Grünwald *et al.* (2002) observed in *Fagus sylvatica* and *Quercus robur* that cell divisions of cambial and ray cells lead to the formation of the callus. In *Abies alba*, *Picea abies*, *Pinus sylvestris* and *Larix decidua*, Oven and Torelli (1999) showed that callus was formed by hypertrophia and hyperplasia of all cambial cells as well as phloem parenchyma. For the 12 species studied here, the parenchyma phloem cells seemed to be the main contributor to the callus formation, which is the first step of each recovery process. Although the cambial zone varied significantly between the dry season and the rainy season for 10 out of the 12 studied species (Fig. 5.2a), the interspecific variation in the thickness of the cambial zone was not very pronounced and could not explain differences in bark recovery. *K. senegalensis*, *L. lanceolata*, *A. africana* and *U. togoensis* showed a similar thickness of the cambial zone (91.5 µm, 79.8 µm, 79.2 µm and 73.5 µm). However *K. senegalensis* had a bark recovery rate significantly different from the other three species: 10.0 cm against 1.2 cm, 0.3 cm and 0.7 cm. This confirmed that the thickness of the cambial zone was not a key-variable to explain the bark recovery rate in the 12 species of this study.

While bark recovery could largely be explained by the amount of phloem parenchyma, xylem parenchyma and fibres were less as half as informative (Table 5.2). In the case of edge regeneration, the wood tissue existing before wounding was mainly concerned with the

process of compartmentalization than the callus formation. Wounding stimulates the parenchyma around the wound towards forming a barrier to protect the living tissue. The barrier can be formed by the production of tyloses and other compounds within vessels, rays or fibres (e.g. Schmitt & Liese 1990, 1993; Clerivet *et al.* 2000; Sun *et al.* 2006). This could be explained by the edge growth as major process of wound repair in the studied species. Sheet growth was poor to nonexistent except for *M. polyandra* and *P. erinaceus* (Delvaux *et al.* 2009). The process of surface callus formation is quite different from that of forming callus on the wound edges. In the case of surface callus formed after debarking, Stobbe *et al.* (2002) observed in *Tilia* sp. that undifferentiated xylem cells at the stage of primary wall formation were involved in callus formation. On the contrary, Li and Cui (1983) suggested that the surface callus formation of *Eucommia ulmoides* is attributed to the repeated partial differentiation of the residual immature xylem and proliferation of ray cells.

Finally, the presence of sclereids in the conducting phloem zone was found to be an interesting anatomical variable although it was poorly correlated with the bark recovery rates (Table 5.2 and Table 5.3). Only in *L. lanceolata*, *M. polyandra* and *U. togoensis*, sclereids were found near the cambial zone (16.7 µm to 104.0 µm) preventing a continuous and thick development of the conducting phloem zone (Fig. 5.5). This was expressed by a thin conducting phloem layer (218.7 µm, 182.6 µm and 323.5 µm) and consequently by a poor edge growth: 1.2 cm, 0.1 cm and 0.7 cm.
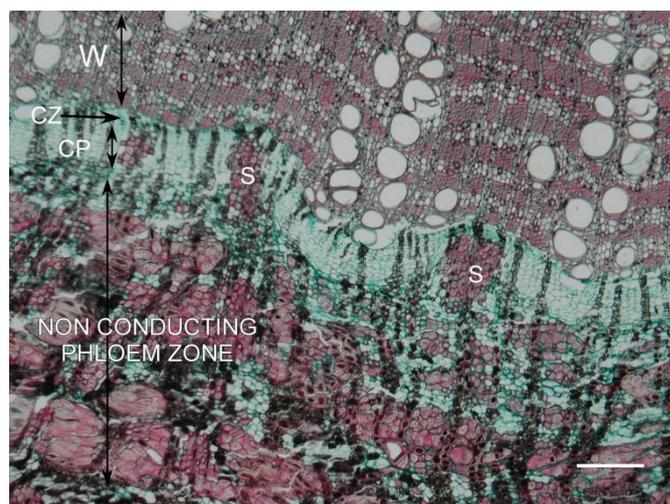
Fig. 5.5: Sclereids (S) in conducting phloem zone of *Lophira lanceolata*. W: Wood Zone, CZ: Cambial zone, CP: Conducting Phloem Zone. Scale bar: 1 mm

In conclusion we propose that bark recovery is pre-determined, at the anatomical level, by the width of conducting phloem. Given that the 12 studied species showed a large range of bark recovery rates (0.1 to 10.0 cm/y), we assume that they are representative of the variety of wound healing responses (i.e. wood and bark tissue production) in a high number of African tree species. Consequently, our results offer the advantage to foresee the potential of wound closure in any trees of which bark could be harvested. The presence of sclereids within the conducting phloem zone is remarkable in *L. lanceolata*, thus further insight in the role of slereids would explain why this species prefer sclereids above good recovery mechanism.

Acknowledgements

C'est la sélection des détails et non pas leur nombre, qui donne à un portrait sa ressemblance.

Alexis Carrel

Il y a des choses que l'intelligence seule est capable de chercher, mais que par elle-même elle ne trouvera jamais. Ces choses, l'instinct seul les trouverait, mais il ne les cherchera jamais.

Henri Bergson

CHAPTER 6

General conclusion
Future Directions

General conclusion

In the framework of this research, we wanted to address the bark regeneration process both from the standpoint of tree ecology and of wood anatomy. We aimed, on the one hand, to determine the best conditions of bark collection, and, on the other hand, to examine the anatomical features which explain the growth mechanism of the new bark. Despite an ever-growing demand for NTFPs (Hamilton 2004; Ticktin 2004), only few studies had been undertaken to propose management measures that meet human needs and at the same time ensure the survival of the tree (Cunningham & Mbenkum 1993; Botha *et al.* 2004b; Geldenhuys 2004; Vermeulen 2006; Geldenhuys *et al.* 2007; Newton 2008; Vermeulen 2009). Since this topic in West Africa has not been studied before, we chose to analyse 12 woody medicinal species sought for their bark. This relatively high species number allowed us to cover the largest possible range of responses to debarking. This was necessary to establish a management model which can be applied to other species in the future.

OBSERVATION – CHOICE – DECISION: THE THREE STEPS OF THE DECISION MODEL

The observation and the measurements of the different reactions of the debarked tree in the natural environment is the first step of the development of the decision model (Fig. 6.1). The second step consists of establishing categories - qualitative and quantitative – for each type of response obtained. The third step is that of the decision about the technique to be applied to each species, in particular in order to ensure a sustainable harvest.

The second chapter enabled us to develop a first decision model, to identify the most adequate debarking technique for each of the 12 species as a function of their responses following bark removal: edge growth (regeneration developing from the edge of the wound), sheet growth (regeneration developing from the surface area of the wound), insect attack, agony shoots vegetative shoots developing around a wound in response to wounding). In the third chapter we refined prescriptions, notably thanks to the biannual study of the bark edge growth pattern for each species. In this way, as a function of the wound area, the time needed to close is estimated. The first classification consists in creating four categories of average bark regeneration rate (edge growth): poor, fair, good and very good. According to its average yearly bark regeneration rate, a category will be chosen for each species. Among the 12 species studied, only five of them showed a good rate of bark regeneration (> 4 cm/year) after bark removal: *K. senegalensis*, *L. kerstingii*, *M. indica*, *P. biglobosa* and *P. kotschyi*. For these five species, a study of the influence of the bark harvest season, of the tree size, and of the collection intensity was carried out. In Fig. 6.1, we only indicate the best regeneration rate per season, by d.b.h., and by intensity, for each species. In the case of *K. senegalensis*, we recommend to collect the bark from big trees (> 30 cm d.b.h.) during the rainy season, on 75% of their circumference (= 75% trunk debarked). As for *M. indica,* this species does not require any particular recommendation, as the growth rate of its new bark is similar whatever the season, tree size and collection intensity. All the species studied present zero or low sheet growth. Therefore, in our model, sheet growth was not incorporated into the decision tree. Nevertheless, in a future study, sheet growth could always be considered and, if needed, the model should be completed to adjust for other conditions. That would be the case if the model was applied to *Quercus suber* and *Eucommia ulmoides*, as both species have a sheet growth capable of covering the whole wound area (Li *et al.* 1982; Li & Cui 1988; Moreira *et al.* 2009). We also did not considered a 100% bark removal (I7) in this model, as this technique does not allow sustainable collection. For all 12 species, 75.9% of the specimens having been submitted to this intensity (100% trunk debarked) died within two years. *M. indica* and *A. africana* survived two years after ring-barking (100%) without any bark regeneration.
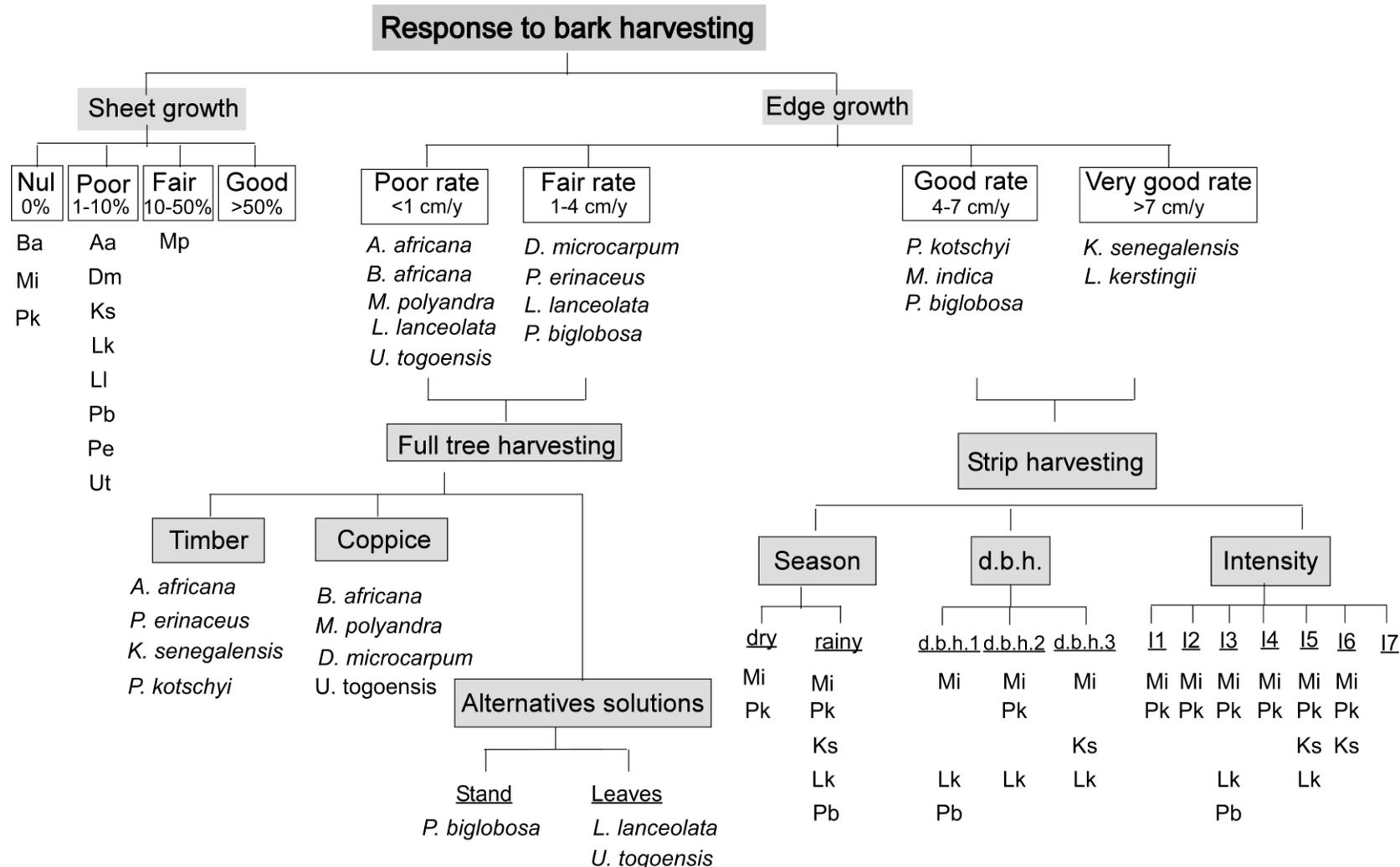
Fig. 6.1: Schematic framework synthesizing the decision model dealing with the medicinal tree species coveted for their bark. The schema illustrates the successive steps needed to provide the most appropriate harvesting system for each species. d.b.h.1: 10-20 cm, d.b.h.2: 20-30 cm, d.b.h.3: >30 cm. Portion of the trunk debarked: I1 = 20% of the trunk circumference, I2 = 2 x 10 %, I3 = 50%, I4 = 2 x 25%, I5 = 20% with square shape, I6 = 75% and I7 = 100%. Ks, *Khaya senegalensis*; Lk, *Lannea kerstingii*; Mi, *Mangifera indica*; Pb, *Parkia biglobosa* and Pk, *Pseudocedrela kotschyi*.

Among the species showing a poor or fair regeneration rate (< 1 cm/y to max 4 cm/y), debarking techniques applied in this research are not considered as desirable and sustainable. Therefore, for *A. africana*, *B. africana*, *M. polyandra*, *L. lanceolata*, *U. togoensis*, *D. microcarpum* and *P. erinaceus*, we suggest a full tree harvesting. Given that some medicinal species are also sought for timber exploitation, the management measure would be to collaborate with the companies which exploit those species, in order to collect the bark before trees are transformed into lumber. This collaboration allows collection of large amounts of bark from *A. africana* and *P. erinaceus*, while avoiding a technique of bark removal harmful to the standing tree. This measure is valid for *K. senegalensis* and *P. kotschyi* which are also logged for commercial purposes. This additional bark supply would allow increasing bark harvest from these two species without exerting an additional debarking pressure on populations in the natural environment. The species having formed agony shoots around the debarked areas show their predisposition to management by coppicing: *B. africana*, *D. microcarpum* and *M. polyandra*. It is thus recommended to cut these species at 1 m from the ground and to collect the bark on the whole length of the trunk, as well as from the branches. For the species which do not present the above criteria, it is proposed to explore new research approches, in order to develop alternative solutions to debarking in the natural environment: planting in clearings or next to villages (agro-forestry system), and harvesting of leaves for the active substance similar to that found in the bark. Among the species studied, the planting solution is adequate for *P. biglobosa* and the use of leaves instead of bark could be adapted to *L. lanceolata* and *U. togoensis*. For these two species, additional chemical studies should be undertaken.

Overall, the obtained intra-specific variability indicates that selection of trees showing the best regeneration characteristics may be a technique suitable to all species.

DEBARKING SEEN THROUGH THE XYLEM VESSELS

Bark removal results in a disturbance of the internal system of the tree, on the one hand by interrupting phloem sap circulation, and on the other hand by modifying the anatomy of the wood produced after the wound. This notably occurs through the well known process of compartimentalisation (Shigo 1984a) which consists, through chemical and anatomical modifications, in protecting the wood already formed from external aggressions, once it has been wounded.

Chapters 2 and 3 allowed us identifying the species which possess a good capacity for regenerating their bark and thereby for resuming wood production. However, measured differences in regeneration rates among the 12 species studied indicate that the factor(s) influencing recovery is (are) more endogenous than exogenous. This first conclusion led us to question the role played by vessels in the process of recovery after debarking (Chapter 4). Some studies have addressed the anatomical changes in vessels after wounds (Aloni & Zimmermann 1984; Rademacher *et al.* 1984; Lev-Yadun & Aloni 1993; Novitskaya 1998; Leal *et al.* 2008), but, to our knowledge, no study sought to explore the spatial impact of debarking within the tree, and the link existing between anatomical modifications in vessels and the capacity of the tree to close its wound.

We can conclude that the disturbance of the specific conductive area, expressed as the sum of vessel area per mm², occurs essentially right at the level of the wound, where the debarking ends and where recovery will start. The whole portion of the tree unaffected by bark removal (variable according to the percentage of debarking) can thus continue its physiological development without stress. Therefore, it was interesting to show that *D. microcarpum*, *K. senegalensis*, *M. indica*, *M. polyandra* and *P. erinaceus,* species which achieve the best wound closure (46 to 77.1% of wound closure cf. Chapter 2), are also those which, two years

after debarking, recovered the same conductive area as that before debarking. As these species live in the same environment, we can exclude the influence of climate or of other external factors to explain the difference in formation of new vessels. The results confirm that it is rather one or several endogenous factors which determine the capacity of a species to react to debarking. It is known that auxin plays a role in vessel formation (Aloni 1992) and notably in diminution of vessel size after a wound (Aloni & Zimmermann 1984; Aloni 1992; Mwange *et al.* 2003; Frankenstein & Schmitt 2006).

CAN BARK RECOVERY BE PREDICTED?

Interest for medicinal plants is increasing and therefore we should be able to give reasonable advice for management of new species which would be submitted to a pressure too high to sustain. This potential pressure, albeit real, led us to approach the field of prediction! Which tissue(s) is (are) the most involved in recovery of the bark when it is lateral? Some studies have demonstrated involvement of different tissues in the healing process, in the days following debarking (Schmitt & Liese 1993; Oven & Torelli 1999; Grünwald *et al.* 2002; Frankenstein *et al.* 2005). But the question arises whether there is an anatomical character which would be more important or more influential than others, and which would help predict that a given species would have the capacity to regenerate its bark laterally, thereby closing the wounded zone? Or, on the contrary, that some species would be assigned to the category "poor rate"? Is this key anatomical character to be found within the xylem, in the phloem or in the vascular cambium? Taking advantage that our study was devoted to 12 species for which data on recovery rates are available (Chapter 3), we collected branches of intact specimens of the same species in order to analyse quantitatively their anatomy (wood, cambium, bark). Our study clearly shows that it is the size of the conducting phloem which explains best bark regeneration. The thickest the conducting phloem zone is, the highest the capacity of the tree to close the wound. *K. senegalensis* and *L. lanceolata* present a contrasted rate of edge growth (10.0 cm/y and 1.2 cm/y, respectively) and they also differ markedly in the size of their conducting phloem: 695.2 µm and 218.7µm. Thus, following a removal of bark down to the wood, the key factor of lateral regeneration is on the bark side, and not on the wood side. Wood remains essentially involved in the compartmentalisation process, which consists in blocking all invasions from the outside, in order to protect the wood not directly in contact with the wounded zone. As for the cambial zone, it plays a role in bark recovery at the time of callus formation (Oven & Torelli 1999; Grünwald *et al.* 2002). However, the size of the cambial zone is not a factor which discriminates the species as far as bark regeneration is concerned.

CONTRIBUTION

Our research topic is not highly developed yet in the scientific community, even though the results are expected with great interest. South Africans (Geldenhuys 2004; Vermeulen 2006; Geldenhuys *et al.* 2007; Vermeulen 2009) were the first to investigate and follow regeneration of bark of medicinal species in Africa, essentially with an emphasis on the ecological aspects and on the management of the problem that may arise from bark collection. By contrast, in Europe (Schmitt & Liese 1993; Oven & Torelli 1994; Grünwald *et al.* 2002; Frankenstein & Schmitt 2006), in Israel (Aloni & Zimmermann 1984; Lev-Yadun & Aloni 1993) and in China (Li & Cui 1988; Cui & Li 2000; Mwange *et al.* 2003), anatomical studies investigated in detail the mechanisms of regeneration after debarking. No previous study combined both

aspects. Nevertheless the approaches are complementary, if one wants to improve the selection of trees with anatomical characters allowing good regeneration capacity.

For many species that are in high demand for medicinal bark, the relevant information on tree response to wounding is not available, and an adaptive management approach would therefore be required (Vermeulen 2009). The 12 medicinal species studied in this research contribute to the set of existing from South Africa, Malawi, Zambia, where no less than 22 species have already been included in an ecological survey of bark recovery (Geldenhuys *et al.* 2007). We have exploited the different options for developing sustainable solutions in the exploitation of non-timber forest products which are medicinal plants. Most often, the NTFPs are collected in the natural environment without specific guidelines, with the well known consequences: disturbance of population structure, of population density, of their reproductive capacity, and of the ecosystem (Cunningham & Mbenkum 1993; Cunningham *et al.* 2002; Ticktin 2004; Gaoue & Ticktin 2008).

The management suggestions we make in this study take into account the characteristics of each species, so that the recommendations are best fitted to the maintenance of these species in the natural environment. For the sake of coherence and continuity, we used as a template the management model developed in South Africa (Vermeulen 2006), that we adapted and completed as a function of our own context and results. Among other things, we determined the time needed, for each species, to close a wound, depending on its yearly regeneration rate. Furthermore we specified the season of collection, tree size and debarking intensity suitable to five species which can be exploited sustainably for their bark: *K. senegalensis*, *L. kerstingii*, *M. indica*, *P. biglobosa* and *P. kotschyi*. In most studies on NTFPs, a small species number was studied and often a single species is analysed. The advantage of having compared 12 species allowed us to get a wide range of responses and, consequently to develop a comprehensive decision model. Complementary studies could aim at testing alternative solutions for those species which are not able to achieve a good level of bark regeneration. In this respect, we have suggested (i) to study the reactions of *B. africana*, *D. microcarpum* and *M. polyandra* to a management by coppicing, (ii) to conduct trials of plantation of *P. biglobosa*, both next to the villages and in forest clearings, and (iii) to test the chemical composition of leaves and bark of *L. lanceolata* and *U. togoensis*. The collaboration with timber companies is not a scientific task, but rather pertains to relations which can be established between them and individuals and societies exploiting the bark of *A. africana*, *K. senegalensis*, *P. erinaceus* and *P. kotschyi*.

An original result of our research is to have shown which anatomical characters favour bark recovery among the 12 species tested. If the recovery processes had been studied before, the variation of their efficiency among different species had not been evaluated. For instance, we could show that, whenever the conductive area after two years was similar to that before debarking, the wound closure was better, thanks to a sufficient production of wood and bark. Moreover, the predictive study carried out through the anatomical analysis of non-debarked specimens taught us that the thickest the zone of conducting phloem, the quickest and the more complete the recovery of bark and wood will be. This pioneer study successfully opened a new way to study bark recovery.

Future directions

As it is often the case in research, the larger impact appears to be that we have raised more questions that we have resolved. Perspectives of further research are presented as a list of questions. My hope is that one or two researchers draw from this list a source for their inspiration.

- *K. senegalensis:* its excellent recovery rates (10.0 cm/y) lead us to search further and improve the understanding of the mechanisms which underlie such a performance. What is the role of auxin in bark regeneration of *K. senegalensis*? Is auxin an additional factor which explains why *K. senegalensis* closes its wounds?
- *L. kerstingii*: this species presents the second best edge growth (9.0 cm/y) but at the same time suffers awful damages from insect attacks, with however a great intra-specific variability with respect to these attacks. What factors favour the resistance of some specimens against insect attacks, while others are mortally affected?
- *M. polyandra* has the best sheet growth. How do certain regeneration islets survive for two years without any contact with other regeneration areas right in the middle of the debarked zone?
- *M. indica* exhibits two peculiarities: (i) it survives for two years after having been debarked on its entire circumference (100% trunk debarked); (ii) it presents the same yearly regeneration rate whatever tree size and debarking intensity. What is the strategy used by this species for maintaining unaltered functioning despite the disturbances that affect it?

Chaque publication scientifique ne sert qu'à poser 10, 20 questions. Chaque découverte scientifique est passionnante parce qu'elle ouvre un univers de questions. Si les questions vous angoissent, ne soyez pas scientifique.

Boris Cyrulnik

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-Où sont les hommes ? Demanda poliment le petit Prince. La fleur, un jour, en avait vu passer une caravane.
-Les hommes ? Il en existe, je crois, six ou sept. Je les ai aperçus il y a des années. Mais on ne sait jamais où les trouver. Le vent les promène. Ils manquent de racines, ça les gêne beaucoup.

<div align="right">Antoine de Saint-Exupery</div>

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SAMENVATTING

Geneeskrachtige planten genieten een groeiende belangstelling vanwege zowel de locale markten als de internationale farmaceutische nijverheid. Actieve bestanddelen worden dikwijls uit de schors gewonnen van bomen in natuurlijke bossen. Met het oog op bosbehoud en om uitputting van enkele doelsoorten te vermijden, is een verantwoord beheer noodzakelijk.

Er is een groot gebrek aan ecologische gegevens over de reactie van bomen op het verwijderen van schors. Het gebrek aan kennis werkt overexploitatie van medicinale planten in de hand. Dit is een ernstige bedreiging voor de duurzame beschikbaarheid van geneesmiddelen voor eerstelijns gezondheidszorg in landelijke gebieden van tropisch Afrika.

Een eerste gedeelte van de dissertatie behandelt de ecologische respons van 12 medicinale boomsoorten na een experimentele schorsoogst op wilde populaties. Omdat hier weinig informatie over beschikbaar is, werden de reacties van de bomen gedurende de twee jaar na de ontschorsing gevolgd. Gebaseerd op inzichten in de respons van bomen op experimentele ontschorsing, werd een beheersstrategie uitgewerkt voor elke soort met behulp van een beslissingsmodel. Het macroscopische fenomeen van schorsherstel is een gevolg van veranderingen op weefselniveau ten gevolge van beschadiging van cellen.

In een tweede gedeelte worden dan ook de anatomische kenmerken voorgesteld die aan de basis liggen van een reactie op ontschorsing. Omwille van hun belangrijke rol voor de boomfysiologie, werd de reactie van vaten op ontschorsing onderzocht. De variabiliteit van de vatkenmerken werd geanalyseerd zowel in ruimte als in de tijd. Bovendien werd het potentieel nagegaan van verschillende anatomische kenmerken om de capaciteit voor schorsherstel te evalueren.

In het ecologische gedeelte van het onderzoek, werd in 2004 schors geoogst van 925 bomen die behoren tot 12 soorten, verdeeld over 38 sites in de lichtbossen ("*woodlands*") van Benin. Bomen werden ontschorst volgens een schema met drie veranderlijken: (i) het oogstseizoen (droog- of regenseizoen), (ii) de afmetingen van de boom (drie klassen van diameter op borsthoogte) en (iii) de ontschorsingsintensiteit (zeven intensiteiten variërend van 20% tot 100 % van de stam). In de loop van een periode van twee jaar werden elke zes maanden op elke boom metingen uitgevoerd om informatie over zijn reactie in te zamelen. Volgende factoren werden geïntegreerd in de analyse: de hergroei van schors, de vegetatieve groei, de gevoeligheid voor insectenaantasting, de overlevingsgraad, de patronen van hergroei, de invloed van het groeiseizoen, de afmetingen van de boom en tenslotte de invloed van de ontschorsingsintensiteit op de hergroei. De anatomische studie omvat twee verschillende analyses. Voor een eerste analyse werd één boom per soort geveld en een stamschijf werd ingezameld ter hoogte van de ontschorsing. Op het kopse vlak van elke schijf werden de vatkenmerken (oppervlakte, densiteit) opgemeten met het beeldanalyseprogramma AnalySIS Pro 3.2. De metingen werden uitgevoerd op drie verschillende plaatsen in radiale richting (voor en na de verwonding) om de invloed van de tijdsfactor van schorsoogst te evalueren. Om de invloed van de ruimtelijke factor na te gaan, werd gemeten op drie verschillende plaatsen op de stamschijf. In een tweede luik van de anatomische studie werden volledige stamschijven van takken ingezameld op niet ontschorste bomen van elk van de 12 soorten. Een totaal van 12 anatomische variabelen werden gemeten in respectievelijk het hout, de cambiale zone en het floeem. Vervolgens werd de correlatie getest tussen de elk van de variabelen en de graad van schorsherstel (data uit hoofdstuk 3).

Uit de halfjaarlijkse waarnemingen blijkt dat enkel *Khaya senegalensis* en *Lannea kerstingii* de volledige wonde overdekken door schorsgroei vanuit de kanten ("edge growth"). Het ander uiterste waren *Afzelia africana*, *Burkea africana*, *Maranthes polyandra* en *Uapaca togoensis* die zeer weinig "*edge growth*" vertoonden. *Maranthes polyandra* had goede schorsgroei vanuit het vlak van de verwonding zelf ("sheet growth"). De andere elf soorten hadden geen of zeer weinig "*sheet growth*". Het ringen van de bomen ("*ring-barking*" of schors

verwijderen over 100% van de stam) bleek geen techniek te zijn die de duurzame exploitatie van een soort mogelijk maakt. Bomen waarvan 75% van de schors verwijderd werd bleven anderzijds wel in leven en vertoonden schorsgroei vanuit de randen van de verwonding. Het patroon in de graad van schorsherstel gedurende de twee jaar van het experiment wees erop dat er voor *Khaya senegalensis*, *Lannea kerstingii*, *Mangifera indica*, *Parkia biglobosa* en *Pseudocedrela kotschii* goede kansen zijn voor een duurzame schorsoogst op voorwaarde dat deze soorten twee jaar de tijd krijgen om de wonde te sluiten. Met het oog op een verhoogde schorsregeneratiegraad van deze soorten werd het meest geschikte seizoen, de optimale diameter op borsthoogte en de beste ontschorsingsintensiteit bepaald. De gevoeligheid voor insectenaantasting was soortafhankelijk. Het aantal boorgangen verschilde duidelijk naargelang van de soort. *D. microcarpum*, *P. biglobosa* en *L. kerstingii* vertoonden de minste weerstand tegen insectenvraat. Bij *P. biglobosa* en zeker bij *L. kerstingii* is het mogelijk dat de schade ten gevolge van insectenaanvallen de stabiliteit van de bomen vermindert en zelfs tot breuk leidt. Op het anatomische vlak blijken de ruimtelijke verschillen in de kenmerken volgend op ontschorsing veel minder belangrijk dan de veranderingen in de tijd. Het herstel van de oorspronkelijke kenmerken van de vaten (grootte en densiteit) na verwonding is een traag proces dat minstens twee jaar vraagt. Anderzijds was op twee centimeter van de wonde geen effect merkbaar in het hout. Er werd voor vijf soorten een positieve correlatie vastgesteld tussen de mogelijkheid om een grote hoeveelheid schors te produceren en de mogelijkheid om het geleidingsweefsel na twee jaar op een initieel niveau te brengen. Onder de 12 anatomische kenmerken die getest werden, was het de dikte van het geleidende floeem dat het best de graad van schorsherstel verklaarde.

De experimentele ontschorsing wees op de complexiteit waarmee beheer te kampen heeft. Er zijn een waaier van factoren die schorshergroei beïnvloeden. Gebaseerd op onze resultaten, ontwikkelden we een beslissingsmodel om het bosbeheer te helpen bij de selectie van de technieken voor de oogst van boomschors, zoals daar zijn hakhout, gebruik van de bladeren i.p.v. de schors, aanplantingen en samenwerking met houtexploitanten. Bovendien is de studie van de graad van schorsherstel een relevante methode om voor elke soort de tijd te bepalen die nodig is om een verwonding te sluiten.

Het is nodig om de capaciteit van de soorten om te reageren op stress beter te begrijpen. In de loop van dit onderzoek hebben we het verband aangetoond tussen de ecologische en de anatomische reacties ten gevolge van ontschorsing. De vaten bleken zeer goede anatomische kenmerken te zijn om de reacties van de bomen op stress te volgen. De studie van vatafmetingen en densiteit hebben mogelijk gemaakt om aan te tonen dat de gevolgen van de ontschorsing beperkt blijven tot de plaats van de verwonding. Het is daarom dan ook begrijpelijk dat het leven van de boom niet in gevaar gebracht wordt, zelfs al wordt 75% van de schors weggenomen. Er werd ook aangetoond dat de dikte van het geleidende floeem een goede indicator is van de potentiële capaciteit van een boom om een verwonding te sluiten.

De twaalf bestudeerde soorten vertoonden veel verschillen in graad van schorsherstel (0,1 tot 10,0 cm per jaar). We gaan van de veronderstelling uit dat ze representatief zijn voor de variabiliteit in wondgenezing (aan de hand van hout en schorsvorming) bij de meeste Afrikaanse boomsoorten. Onze resultaten bieden dan ook het voordeel om het potentieel van wonddichting te voorspellen van de soorten die in aanmerking komen voor schorsoogst.

SUMMARY

The growing interest for medicinal plants from both international industry and local markets requires management of tree bark harvesting from natural forests in order to prevent inappropriate exploitation of target species. The lack of ecological data concerning the responses of tree species to bark harvesting often leads to the overexploitation of medicinal tree species, threatening the essential source of medicines for primary health care of rural populations in Africa. Although the importance of bark was proved daily by its local uses, no anatomical studies were carried out to explain the variety of responses following bark harvesting observed in medicinal tree species all over Africa.

The first part of this work presents the ecological responses of 12 medicinal tree species after an experimental bark harvesting carried out on natural populations in the wild. Reactions of trees over a two-year period following bark harvesting are relatively unknown until now. Based on various responses of trees following experimental debarking, a management strategy is elaborated for each species through a decisional model. The macroscopic phenomenon of bark recovery is the expression of tissue modification in reaction to injury at the microscopic level. The second part presents the anatomical features underlying the reaction to bark harvesting. Given the important role of vessels in tree physiology, the temporal and spatial impact of bark harvesting on anatomical changes of vessels are investigated. Moreover the potential of several anatomical variables to predict the capacity to re-grow after bark harvesting is tested.

For the ecological part, bark was harvested from 925 trees belonging to 12 species in 38 woodland sites in Benin in 2004. Trees were debarked following a combination of three factors: (i) season of bark harvesting (during the dry season or during the rainy season), (ii) size class of the tree (three classes of d.b.h.) and (iii) intensity of debarking (seven intensities ranging from 20% to 100% of trunk debarked). Every six months over the course of the two-year experiment, measurements were made on each tree to collect information on their reactions: edge growth (regeneration developing from the edge of the wound), sheet growth (regeneration on the surface of the wound), vegetative growth, sensitivity to insect attacks, survival, patterns of re-growth and influence of season, tree size and intensity of debarking on re-growth. The anatomical study includes two different analyses. First, two years after debarking, 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 and density) were measured with the image analysis software AnalySIS Pro 3.2. The measurements were made on three locations in the radial direction (before and after wounding) to evaluate the temporal impact of bark harvesting and on three locations around the disc to evaluate the spatial impact of bark harvesting. Second, discs of branches were collected on non-debarked trees of the 12 species. A total of 12 anatomical variables were measured in wood, cambium and phloem zone and thus the correlation was tested between the bark recovery rate (data obtained from chapter 3) and each variable.

Over the two-year study, only *Khaya senegalensis* and *Lannea kerstingii* showed complete wound recovery by edge growth. At the other extreme, *Afzelia africana*, *Burkea africana*, *Maranthes polyandra* and *U. togoensis* had poor edge growth. *Maranthes polyandra* showed good sheet growth, whereas the other 11 species had none or poor sheet growth after total bark harvesting. Ring-barking (100% of trunk debarked) did not appear to allow a sustainable exploitation of any species, while all trees with 75% of debarked circumference remained alive and produced edge growth. The biannual pattern of bark recovery rate indicated that *K. senegalensis*, *L. kerstingii*, *Mangifera indica*, *Parkia biglobosa* and *Pseudocedrela kotschyi* were potentially able to support a sustainable bark harvesting. For the last three species, the condition for wound closure was a delay longer than two years. To focus on these species' higher bark regeneration rate, the best season, d.b.h. and intensity were determined. Sensitivity to insects was species-specific. The number of insect holes was clearly species-

dependent. *D. microcarpum*, *P. biglobosa* and *L. kerstingii* showed the least resistance to insect attacks. However, in *P. biglobosa* and especially in *L. kerstingii*, the damages inflicted by insects may weaken the stability of trees and eventually lead to cracking. At the anatomical level, spatial changes in the wood features after bark harvesting damage were much less important than temporal changes. Recovery of the vessel features (size, density) towards their condition before wounding is a slow process that requires at least two years to complete. On the other hand, at two cm from the bark harvesting wound the wood was not affected. The positive correlation found for five species between the ability to produce a high amount of new bark and the ability to recover the initial conductive area two years after wounding helps understanding the anatomical adaptation allowing good bark regeneration. Among the 12 anatomical variables tested, the thickness of the conducting phloem zone emerged as the most important one for to the bark recovery rate.

This experimental bark stripping demonstrated the complexity of the management due to the variety of factors influencing bark re-growth. Based on our results, we developed a decisional step method to help forest managers to select the best techniques for managing medicinal tree species. We also proposed an alternative for species that cannot sustain bark harvesting, e.g. coppice management, harvesting leaves instead of bark, stand establishment or collaboration with timber companies. Moreover studying the patterns of bark recovery rate is a pertinent management tool to determine for each species the necessary delay to close a specific wound area. The ability of species to respond to this particular stress deserved better understanding. For that purpose we explored the link between ecological and anatomical reactions to bark harvesting. Vessels appeared as very good anatomical indicators of the tree's reactions to stress. The study of their size and density notably demonstrated that the impact of bark-stripping is limited to the exact place of wounding, with the consequence that a harvest of up to 75% of the trunk does not endanger the tree's life. The width of conducting phloem was also proved to be a predictive indication of the tree's ability to close a wound. Given that the 12 studied species showed a large range of bark recovery rates (0.1 to 10.0 cmy^1), we assume that they are representative of the variety of wound healing responses (i.e. wood and bark tissue production) in a high number of African tree species. Consequently, our results offer the advantage to foresee the potential of wound closure in any tree from which bark could be harvested.

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2000	"Corso introduttivo all'Etnomedicina". Dipartimento di Scienze Antropologiche, Universita degli Studi di Genova, Italy.
1999	Formation in "Ethnopharmacologie Appliquée – Pharmacopées traditionnelles et plantes médicinales". Unité de Formation et de Recherche – Sciences Fondamentales et Appliquées de l'Université de Metz, France.
1989-1993	Master in Botanical Sciences at the Université Libre de Bruxelles with High Distinction.
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ANNEXES

Annexe 1

Annexe 1: Review of litterature on traditional uses, activity and active compounds of medicinal plants focused on the 12 species of this study

Species	Family	Study	Active compound	Disease terminology/ activity	References
Afzelia africana	Fabaceae (C)	E		external wound hypertension chest ache stomach ache febrifuge gastrointestinal stimulant leprosy	(Berthaut 1979b)
		E		epilepsy Intercostal neuralgia dysmenorrhea hyperthermal convulsion oedema	(Adjanohoun *et al.* 1989)
		P		trypanosomiasis	(Atawodi *et al.* 2002)
		E		hypertension constipation febrifuge gastrointestinal stimulant skin wound	(Arbonnier 2004)
		E		malaria stress weakness	(Bockx 2004)
		P		trypanocidal activity against *Trypanosoma brucei brucei*	(Atawodi 2005)
		P, C	tannins alkaloids flavonoids saponins	antibacterial activity	(Akinpelu *et al.* 2008)
Burkea africana	Fabaceae (C)	P, C	tannin	fish poison septic sores	(Watt & Breyer-Brandwijk 1962)
		C	β-sisterol tryptamine		(da Silva & Paiva 1971)

			harman-type alkaloids		
Burkea africana	Fabaceae (C)	C	β-sisterol tryptamine harman-type alkaloids		(Ferreira 1972, 1973a, 1973b)
		P		weak activity against *Staphylococcus aureus*	(Delaveau *et al.* 1979)
		E		cough migraine Arrow poison	(Berthaut 1979b)
		E		migraine oedema diarrhoeas intestinales fever epilepsy	(Adjanohoun *et al.* 1989)
		E		headache migraine dizziness pain inflammation and thrush antineuralgic wound-healing tooth-cleaning agent	(Mathisen *et al.* 2002)
		P, C	proanthocyanidins fisetinidol epicatechin catechin	anti-oxydant and radical scavenging activity	(Mathisen *et al.* 2002)
		E		malaria stomach ache maggot infection malediction (curse) epilepsy diarrhoea	(Bockx 2004)
		E		cough	(Arbonnier 2004)

				migraine	
Detarium microcarpum	Fabaceae (C)	E		diarrhoea haemorrhoids gonorrhoea	(Berthaut 1979b)
		E		analgesics haemorrhoids	(Adjanohoun *et al.* 1985)
		E		haemorrhoids intestinal worms dysmenorrhea oedema	(Adjanohoun *et al.* 1989)
		E		diuretic property inflammation parasite infection	(Iwu 1993)
		C	Lactone fructose glucose sucrose inositol, Pinitol bornesitol		(Abreu & Relva 2002)
		E		diarrhoea amoebiasis haemorrhoids rheumatism	(Arbonnier 2004)
		E		diarrhoea	(Bockx 2004)
Khaya senegalensis	Meliaceae	E	tannin	febrifuge malaria	(Watt & Breyer-Brandwijk 1962)
		E		abortive stomach ache stimulant febrifuge intestin ache dermatitis	(Berthaut 1979d)

				astringent	
				styptic	
				suspension of menstrual period	
				wound	
				ulcer	
				maggot infection	
				malaria	
Khaya senegalensis	Meliaceae	E		dermatitis	(Adjanohoun *et al.* 1989)
				diarrhoea	
		E		sedative and reduced locomotor activity	(Iwu 1993)
		C	three new limonoids		(Olmo *et al.* 1996)
		P		topical antiinflammatory activity	(Lompo *et al.* 1998)
		P		stimulatory impact on lymphocyte cells	(El Tahir *et al.* 1998)
		P, C	limonoid	moderate antiprotozoal activity	(Khalid *et al.* 1998)
		P, C	rearranged limonoid	strong antisickling activity	(Fall *et al.* 1999)
		P, C	limonoid	indirect immunostimulating principle	(Kayser & Abreu 2001)
		P, C	khayanolides A, B, C	antifeedant activity against *Spodoptera littoralis*	(Abdelgaleil *et al.* 2001)
		E		trypanosomiasis	(Atawodi *et al.* 2002)
		P		trypanocidal activity against *Trypanosoma brucei brucei* and *Trypanosoma congolense*	(Atawodi 2003)
		E		anaemia	(Bockx 2004)
				malaria	
				fever	
				maggot infection	
				diarrhoea	
				stomach ache	
				weakness	
				development of the baby	
		E		maggot infection	(Arbonnier 2004)
				malaria	
				fever	
				jaundice	

				diarrhoea	
				anaemia	
				dermatitis	
Khaya senegalensis	Meliaceae	E		external and internal wounds	(Kone *et al.* 2004)
				diarrhoea	
				dysentery	
		P		trypanocidal activity against *Trypanosoma brucei brucei*	(Wurochekke 2004)
		P		trypanocidal activity against *Trypanosoma brucei brucei*	(Atawodi 2005)
		P, C	polyphenols	antioxidant and antibacterial activities	(Karou *et al.* 2005)
		E		malaria	(Ahua *et al.* 2007)
				stomach ache	
		E		skin infection	(Kubmarawa *et al.* 2007)
		P, C	saponins	antimicrobial activity against diarrhea	(Kubmarawa 2008)
			phenols		
			tannins		
		P, C	flavonoids	antimicrobial activity (against *Staphylococcus aureus*,	(Makut *et al.* 2008)
			steroids	*Streptococcus faecalis* and *Candida*	
			tannins	*albicans*)	
		P		antibacterial activity (*Staphylococcus aureus* *Streptococcus pyogenes* *Salmonella typhi Shigella dysenteriae Klebsiella pneumoniae* and *Pseudomonas aeruginosa*)	(Sale *et al.* 2008)
		P		trypanocidal activity against	(Ibrahim *et al.* 2008)

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				Trypanosoma brucei brucei	
Khaya senegalensis	Meliaceae	C	saponins glucosides flavoids volatil oil		(Kubmarawa 2008)
Lannea kerstingii	Annacardicaceae	E		maggot infection	(Ake Assi *et al.* 1980)
		E		development of baby convulsions infantiles fracture	(Adjanohoun *et al.* 1989)
		E		diarrhoea maggot infection development of baby malaria anaemia	(Bockx 2004)
		E		maggot infection diarrhoea oedema epilepsy	(Arbonnier 2004)
		P		trypanocidal activity against *Trypanosoma brucei brucei*	(Atawodi 2005)
Lophira lanceolata	Ochnaceae	C	biflavonoids tetraflavonoids		(Ghogomu *et al.* 1987)
		E		jaundice development of baby	(Adjanohoun *et al.* 1989)
		E		fever jaundice cough bronchitis	(Arbonnier 2004)
		E		menstrual period	(Bockx 2004)
		P		antimicrobial activity	(Akinpelu & Onakoya 2006)
		E		cure for stomach ache skin disease	(Fariku & Kidah 2008)
Mangifera indica	Annacardiaceae	E		astringent dysentery	(Berthaut 1979a)

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				gonorrhea	
				rheumatism	
				fever	
				scabies	
				skin wound	
				vaginal discharge	
				uterus haemorrhage	
Mangifera indica	Annacardiaceae	E		cough	(Ake Assi *et al.* 1980)
				diarrhoea	
		E		anaemia	(Adjanohoun *et al.* 1989)
				hypotension	
				abdominal ache	
				diabetes	
				stomatitis	
		P		antiviral (anti-herpex simplex virus activity)	(Zheng & Lu 1990)
		P		antiviral	(Guha *et al.* 1996)
		P	mangiferin	antitumor, immunomodulatory and anti-HIV effect	(Guha *et al.* 1996)
		P		antiplasmodial and antipyreitc	(Awe *et al.* 1998)
		P		antioxidant	(Sanchez *et al.* 2000)
		P		antioxydant	(Martinez *et al.* 2000)
		P		antioxydant	(Sanchez *et al.* 2000)
		P		antiviral (anti-herpex simplex virus activity)	(Yoosook *et al.* 2000)
		P		antiamoebic and spasmolytic activities	(Tona *et al.* 2000)
		P		antioxydant	(Moreira *et al.* 2001)
		P		anti-inflammatory analgesic	(Garrido *et al.* 2001)
		P		immunomodulatory	(Garcia *et al.* 2002; Leiro *et al.* 2004) (Makare *et al.* 2001)
		P		antioxidant	(Muruganandan *et al.* 2002; Yoshikawa *et al.* 2002; Garrido *et al.* 2004)
		P		antiallergic and anthelmintic activities	(Garcia *et al.* 2003)

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Mangifera indica	Annacardiaceae	P		anti-inflammatory activity	(Garrido *et al.* 2004)
		P		antitumor	(Leiro *et al.* 2003; Sarkar *et al.* 2004)
		E		malaria yellow-fever weakness fever cold stomach ache	(Bockx 2004)
		E		diarrhoea dysentery skin wound rheumatism	(Arbonnier 2004)
		P		vascular modulatory activity	(Beltran *et al.* 2004)
		P		protection against oxidative stress in elderly humans	(Pardo-Andreu *et al.* 2006)
		E		toothache gastrointestinal disorders dynsentery diarrhoea sore gums sore throats	(Akinpelu & Onakoya 2006)
Maranthes polyandra	Chrysobalanaceae	E		healing	(Ake Assi *et al.* 1980)
		E		diarrhoea	(Bockx 2004)
		E		fever wound fracture	(Arbonnier 2004)
Parkia biglobosa	Fabaceae (M)	E	tannins	stimulant toothache mouthwash filariasis gonorrhea bilharzia bronchitis pneumonia	(Berthaut 1979b)

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Annexe 1

				sterility dermatitis leprosy	
Parkia biglobosa	Fabaceae (M)	E		hypertension haemorrhoids dermatitis	(Adjanohoun *et al.* 1989)
		P		analgesic antiinflammatory activity	(Kouadio *et al.* 2000)
		P		antisnake venom activity	(Asuzu & Harvey 2003)
		E		astringent maggot infection stomach ache pregnant woman diarrhoea	(Bockx 2004)
		E		diarrhoea amoebiasis pneumonia bronchitis fever hypertension wound leprosy	(Arbonnier 2004)
		P		piscidial bark	(Fafioye 2004)
		P	sterols triterpenes tannins saponosides anthocyanidins coumarins flavonoids reducing compounds	antibacterial activity against *Staphylococcus aureus*	(Millogo-Kone *et al.* 2007, 2008)
Pseudocedrela kotschyi	Meliaceae	E		digestive troubles stomach ache scabies	(Berthaut 1979d)

			fish poison	
Pseudocedrela kotschyi	Meliaceae	E	incontinence convulsion	(Adjanohoun *et al.* 1989)
		P	trypanosomiasis	(Atawodi *et al.* 2002)
		E	maggot infection malaria absces stomach ache	(Bockx 2004)
		E	fever wound scabies stomach ache diarrhoea	(Arbonnier 2004)
Pterocarpus erinaceus	Fabaceae (P)	E	dysentery wound mouthwash toothache cough stimulant chronic ulcer abortive	(Berthaut 1979c)
		E	urethritis hematuria	(Adjanohoun *et al.* 1985)
		E	anaemia dysmenorrhea development of baby dysentery amenorrhea	(Adjanohoun *et al.* 1989)
		E	fever malaria stomach ache anaemia ininfertility sprain menstrual period	(Bockx 2004)

			diarrhoea	
Pterocarpus erinaceus	Fabaceae (P)	E	wound cough anaemia diarrhoea astringent	(Arbonnier 2004)
		P	triterpenoid two isoflavones	contraceptive agent	(Benie & Thieulant 2004)
Uapaca togoensis	Euphorbiaceae	E	malaria weakness fever stomach ache	(Bockx 2004)
		E	jaundice	(Arbonnier 2004)

(C): Caesalpinioideae; (M): Mimosoideae; (P): Papilionoideae
E: Ethnobotanical study; P: Pharmaceutical study; C: Chemical study

Responses to bark harvesting

Annexe 2

Annexe 2: Bark description of the 12 species focused on this study

Species	Bark description	thickness of the bark
A. africana	grey to dark grey, scaly, layer above: pink to light brown	1.5 ± 0.4
B. africana	grey to black, corrugated, layer above: purplish to brown	1.6 ± 0.4
D. microcarpum	light grey to black, smooth and scaly, layer above: reddish-brown	1.5 ± 0.4
K. senegalensis	mottled grey and brown, smooth but exfoliating in scales, layer above: pink	1.4 ± 0.2
L. kerstingii	silvery-grey, smoothly lightly fissured with a pronounced spiral twist, layer above: pink and white striped	1.8 ± 0.4
L. lanceolata	grey to light brown, coarsely flaking in smal pieces (brittle), layer above: yellow and crimson-red beneath	1.7 ± 0.4
M. indica	grey, smoothly, layer above: white to light yellow	1.3 ± 0.2
M. polyandra	dark grey, square fissured, layer above: red	1.2 ± 0.4
P. biglobosa	grey, fissured in irrelugar small pieces, layer above: orange to red	1.5 ± 0.3
P. kotschyi	grey, deeply fissured, layer above: red	1.8 ± 0.4
P. erinaceus	greyish-brown to dark grey, deeply and reticulately fissured, rough, layer above: dark red	1.5 ± 0.3
U. togoensis	dark grey, deeply cracked, layer above: red	1.8 ± 0.3

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The End

La meilleure des joies est celle que vous procurent les résultats de vos propres efforts.

Idriss Daouda