Annotating PDFs for eReturn

version 1.0; August 28, 2007; full release

1. Introduction

eProof files are self-contained PDF documents for viewing on-screen and for printing. They contain all appropriate formatting and fonts to ensure correct rendering on-screen and when printing hardcopy. DJS sends eProofs that can be viewed, annotated, and printed using the free version of Acrobat Reader 7 (or greater). These eProofs are "enabled" with commenting rights, therefore they can be modified by using special markup tools in Acrobat Reader that are not normally available unless using the Standard or Professional version.

The screen images in this document were captured on a PC running Adobe Acrobat Reader version 8.1.0. Though some of the images may differ in appearance from your platform/version, basic functionality remains similar. At the time of this writing, Acrobat Reader v8.1.0 is freely available and can be downloaded from: http://www.adobe.com/products/acrobat/readstep2.html

2. Comment & Markup toolbar functionality

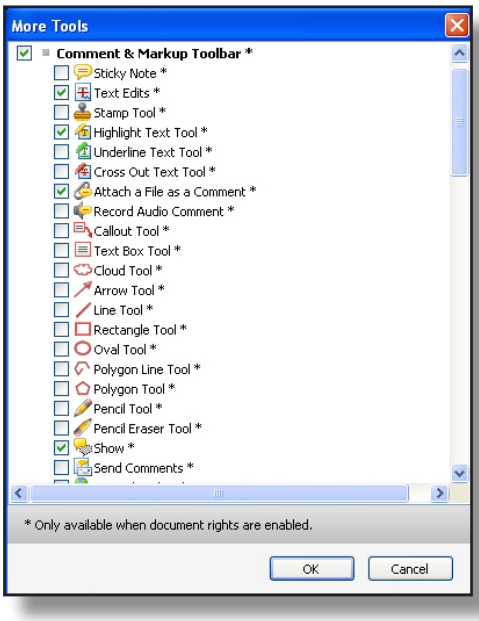
A. Sticky Note tool; B. Text Edits tool; C. Stamp tool; D. Highlight Text tool; E. Callout tool; F. Text Box tool; G. Various Object tools; H. Pencil tool

A. Show the Comment & Markup toolbar

The Comment & Markup toolbar doesn't appear by default. Do one of the following:

- Select View > Toolbars > Comment & Markup.
- Select Tools > Comment & Markup > Show Comment & Markup Toolbar.
- Click the Review & Comment button in the Task toolbar, and choose Show Comment & Markup Toolbar.

To add or remove tools for this toolbar, right-click the toolbar and select the tool. Or, select Tools > Customize Toolbars.



Choose Tools > Customize Toolbars to remove unnecessary items from the toolbar (see Section 7 for suggested toolbar layout)

B. Select a commenting or markup tool

Do one of the following:

- Select a tool from the Comment & Markup toolbar.
- Select Tools > Comment & Markup > [tool].

Note: After an initial comment is made, the tool changes back to the Select tool so that the comment can be moved, resized, or edited. (The Pencil, Highlight Text, and Line tools stay selected.)

C. Keep a commenting tool selected

Multiple comments can be added without reselecting the tool. Select the tool to use (but don't use it yet).

- Select View > Toolbars > Properties Bar.
- Select Keep Tool Selected.

3. The Properties bar

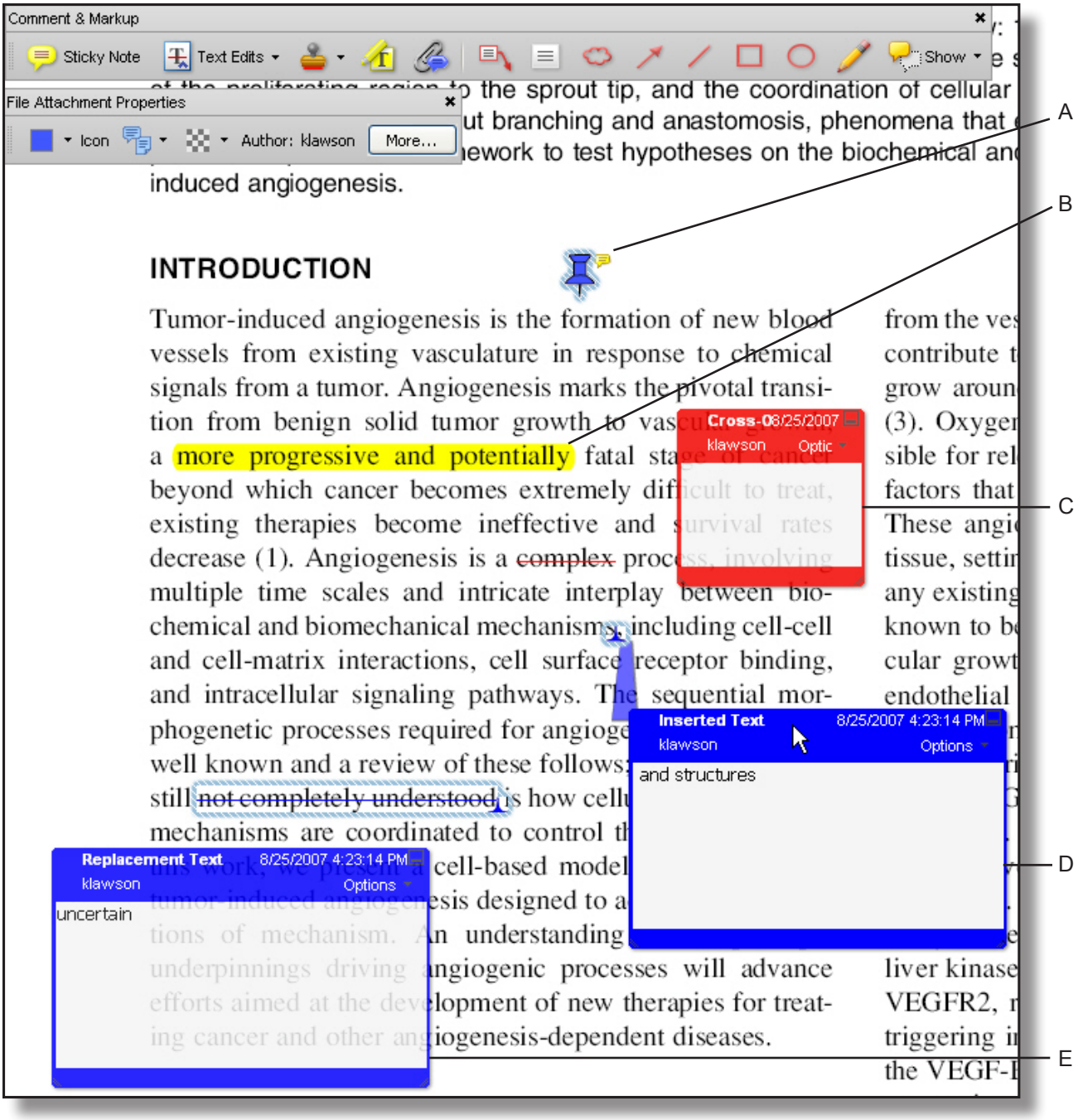
The Properties bar can be used to format text and select options for individual tools.

To view the Properties bar, do one of the following:

- Choose View > Toolbars > Properties Bar.
- Right-click the toolbar area; choose Properties Bar.
- Select [Ctrl-E]

4. Using the comment and markup tools

To *insert*, *delete*, or *replace* text, use the **Text Edits** tool. Select the Text Edits tool, then select the text with the cursor (or simply position it) and begin typing. A pop-up note will appear based upon the modification (e.g., inserted text, replacement text, etc.). Use the Properties bar to format text in pop-up notes. A pop-up note can be minimized by selecting the ⊟ button inside it.



A. Attached file; B. Highlighted text; C. Crossed-out (strike-through) text; D. Inserted text; E. Replaced text

5. Inserting symbols or special characters

An 'insert symbol' feature is not available for annotations, and copying/pasting symbols or non-keyboard characters from Microsoft Word does not always work. Use angle brackets < > to indicate these special characters (e.g., <alpha>, <beta>).

6. Editing near watermarks and hyperlinked text

eProof documents often contain watermarks and/or hyperlinked text. Selecting characters near these items can be difficult using the mouse alone. To edit an eProof which contains text in these areas, do the following:

- Without selecting the watermark or hyperlink, place the cursor near the area for editing.
- Use the arrow keys to move the cursor beside the text to be edited.
- Hold down the shift key while simultaneously using arrow keys to select the block of text, if necessary.
- Insert, replace, or delete text, as needed.

7. Summary of main functions

Insert text - Use Text Edits tool (position cursor and begin typing)
Replace text - Use Text Edits tool (select text and begin typing)
Delete text - Use Text Edits tool (select text and press delete key)
Highlight text - Use Highlight Text tool (select text)
Attach a file - Use the Attach a File with Comment tool (select tool, position cursor and click mouse, select file)

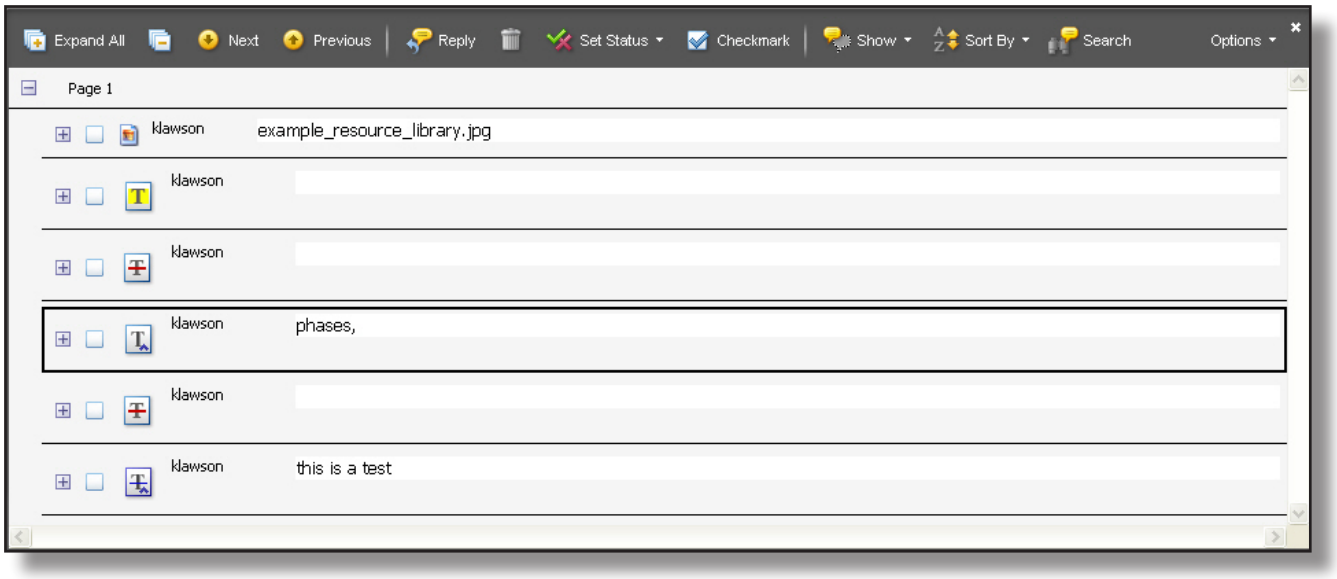
Suggested toolbar layout

8. Reviewing changes

To review all changes, do the following:

- Select the Show button on the Comment & Markup toolbar.
- Select Show Comments List.

Note: *Selecting a correction in the list will highlight the corresponding item in the document, and vice versa.*



Use the Comments list to review all changes

9. The eReturn process

A. An email is received that contains a link to the eProof of the article:
http://eproofing.dartmouthjournals.com/pdfproofing/journal1234.pdf

B. Click on the link to open the proof with the internet browser. Select "Save As" from the browser's 'File' menu to save a copy of the PDF to the desktop or other folder.

C. Close the browser and open the saved PDF file with Acrobat.

D. Make corrections using Acrobat's Comment & Markup tools.

E. Save the PDF file, now with annotations, and return according to the instructions provided by the DJS journal manager.

American Journal of Botany 96(8): 1–10. 2009.

What is disjunctive xylem parenchyma? A case study of the African tropical hardwood *Okoubaka aubrevillei* (Santalaceae)[1]

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The morphological variation and structure–function relationships of xylem parenchyma still remain open to discussion. We analyzed the three-dimensional structure of a poorly known type of xylem parenchyma with disjunctive walls in the tropical hardwood *Okoubaka aubrevillei* (Santalaceae). Disjunctive cells occurred among the apotracheal parenchyma cells and at connections between axial and ray parenchyma cells. The disjunctive cells were partly detached one from another, but their tubular structures connected them into a continuous network of axial and ray parenchyma. The connecting tubules had thick secondary walls and simple pits with plasmodesmata at the points where one cell contacted a tubule of another cell. The imperforate tracheary elements of the ground tissue were seven times longer than the axial parenchyma strands, a fact that supports a hypothesis that parenchyma cells develop disjunctive walls because they are pulled apart and partly separated during the intrusive growth of fibers. We discuss unresolved details of the formation of disjunctive cell walls and the possible biomechanical advantage of the wood with disjunctive parenchyma: the proportion of tissue that improves mechanical strength is increased by the intrusive elongation of fibers (thick-walled tracheids), whereas the symplastic continuum of the parenchyma is maintained through formation of disjunctive cells.

Key words: disjunctive cell wall; *Okoubaka aubrevillei*; Santalaceae; wood formation; wood structure; xylem parenchyma.

The parenchyma in the secondary xylem of woody plants has important functions for the metabolism and storage of reserve substances, as well as for defense against pathogens (Sauter, 1988; Schmitt and Liese, 1990; Carlquist, 2001). Various hypotheses about the role of the parenchyma in the hydraulic function of xylem have been suggested and discussed, in particular, in long-distance water transport and refilling of embolized conduits (Braun, 1984; Pate and Jeschke, 1995; Salleo et al., 1996; Holbrook and Zwieniecki, 1999; Tyree et al., 1999; De Boer and Volkov, 2003; Hacke and Sperry, 2003; Clearwater and Goldstein, 2005). The structure of xylem parenchyma greatly varies among species, and it has been classified morphologically (Kribs, 1937; Metcalfe and Chalk, 1989; IAWA, 1989; Carlquist, 2001). Disjunctive xylem parenchyma is one of the categories that to date has received little attention.

Parenchyma cells and tracheids with disjunctive cell walls occurring in association with large-diameter vessel elements have been mentioned in the literature since the 1930s (Esau, 1965b, p. 262; Fahn, 1990). Currently, xylem (ray and axial) parenchyma with disjunctive cell walls is used as one of the characters in comparative wood anatomy, namely, feature number 113 in the International Association of Wood Anatomists (IAWA) list of microscopic features for hardwood identification (IAWA, 1989). On the basis of histological observations by light microscopy, disjunctive xylem parenchyma has been defined as axial or radial parenchyma cells partly becoming disjunct during the differentiation process but the contact between the cells is maintained through tubular connections (Normand, 1972; IAWA, 1989). Although this definition describes the appearance of disjunctive cells, the occurrence of tubules and identification of disjunctive cells is not easily discernible with a traditional light microscope, and available data about this wood anatomical character are scarce. To our knowledge, there are neither detailed studies nor clear illustrations of the morphology of the disjunctive xylem parenchyma cells, and the mechanism of formation of disjunctive walls is still a puzzling phenomenon in wood formation. Consequently, not only the structure but also the ontogeny and phylogeny of xylem with such characteristics have yet to be studied.

In temperate regions, disjunctive xylem parenchyma has been observed among ray cells in some species of the families Buxaceae, Cornaceae, Ericaceae, and Rosaceae (Richter and Dallwitz, 2000) and among axial parenchyma in *Actinidia* sp. (P. Kitin and S. Noshiro, personal observations). The list of species with disjunctive parenchyma might be longer, but disjunctive cells in temperate woods are rarely reported, probably because this type of structure is not well known and the disjunctions between the cells are typically small and difficult to notice. By contrast, disjunctive parenchyma is a common feature in species of the *Sophora* group of the legumes (Fabaceae) (Fujii et al. 1994) and is apparent in many tropical species (Richter and Dallwitz, 2000; H. Beeckman and P. Kitin, personal observations). Axial parenchyma cells frequently have disjunctive walls in the stem wood of *Okoubaka aubrevillei* Pellegr. et Normand (Santalaceae), which we selected for investigation.

Okoubaka aubrevillei is a fast-growing tree species from the rainforests of West and Central Africa (Tailfer, 1989). Its wood

[1] Manuscript received 21 October 2008; revision accepted 9 March 2009.

The authors thank the late professor Jun Ohtani for his valuable advice on the SEM work and Dr. Barbara Lachenbruch for discussion and critical reading of the manuscript. This work was supported by a grant from the Japanese Society for the Promotion of Science (JSPS) and a Marie Curie Outgoing International Fellowship of the European Commission (MOIF 040400 to P.K.).

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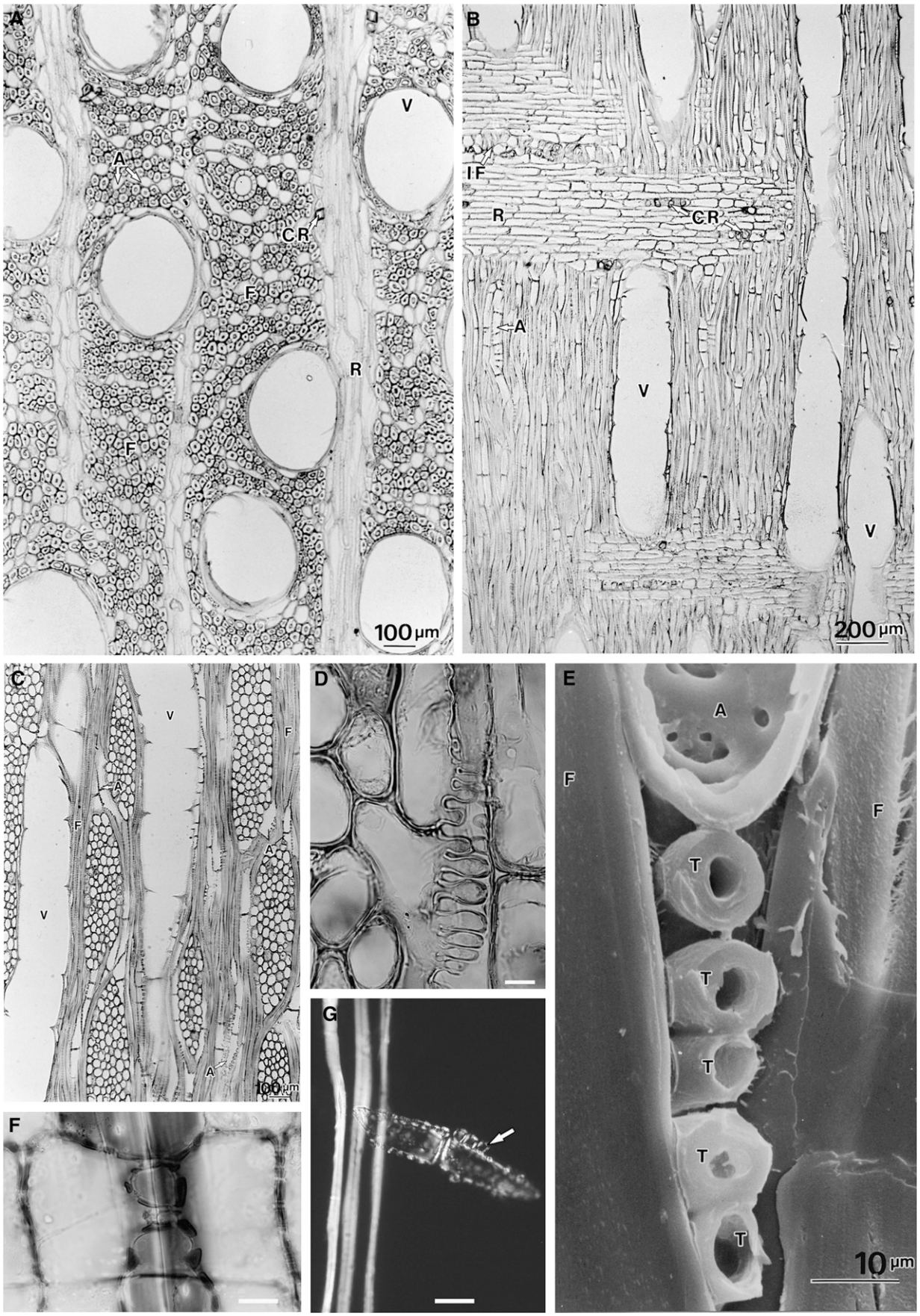
doi:10.3732/ajb.0800355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is straight-grained, heavy, and hard, with a basic density of 0.68 g/cm^3 (Bluskova et al., 1995). Anatomical descriptions of xylem of this species have been reported by Normand (1944, 1950, 1972), Lebacq and Dechamps (1964, 1967), and Normand and Paquis (1977); however, only Normand (1972) noted the occurrence of disjunctive parenchyma.

In this study, our goal was to characterize the three-dimensional (3D) morphology and arrangement of the xylem parenchyma of *O. aubrevillei*. Our hope was to then use these analyses for clues to understanding the pattern of development of disjunctive cells and the factors triggering their formation.

MATERIALS AND METHODS

Plant materials—Wood blocks from six trees of *Okoubaka aubrevillei* Pellegr. et Normand were obtained from xylarium samples of the Royal Museum for Central Africa, Tervuren, Belgium (specimens: Tw 7135, Tw 7967, Tw 8498, collected by R. Dechamps; Tw 5323, Tw 6577, Tw 21883, collected by Exforka [Exploitation forestière au Kasaï]). Only wood sample Tw 8498 has a detailed herbarium voucher; it was collected in Kasai Oriental (Bana-Longo), Democratic Republic of Congo (longitude 21°30′E, latitude 04°48′N, altitude 535 m a.s.l.), in a table land forest. It was a large tree with buttresses (height 20.4 m, main stem length 17 m, diameter 78 cm). The local names of the species are tsiluba, didwe, and mutshi ditoke.

Scanning electron microscopy (SEM)—Small blocks of wood from each of the six trees were cut to 7 mm (longitudinal direction) × 2 mm (radial) × 5 mm (tangential) or 7 mm (longitudinal direction) × 5 mm (radial) × 2 mm (tangential) and rinsed in water. The radial and the tangential surfaces were planed with a razor blade. Then the samples were processed for scanning electron microscopy (SEM) as described by Ohtani et al. (1992) and examined with a scanning electron microscope (JSM-35CFII, JEOL, Tokyo, Japan) at an accelerating voltage of 15 kV.

Conventional light microscopy and transmission electron microscopy (TEM)—Some of the wood samples were trimmed with a razor blade into small blocks with longitudinal and transverse sides of 4–5 mm and were treated with aqueous potassium permanganate or a 1% aqueous solution of osmium tetroxide for 1 h to stain cell walls and improve the contrast of TEM imaging of lignified walls and remnants of membranes and protoplast. Next, the samples were embedded in Epon 812 (TAAB, Berkshire, UK). The epoxy-embedded samples were cut into serial, transverse, semithin (1–5 μm) sections with an ultramicrotome (Ultracut; Reichert-Jung, Vienna, Austria) using a glass knife, and then stained with safranin in a 1% aqueous solution. After washing in water, the sections were transferred onto microscope glass slides and air-dried. Finally, the sections were mounted with mounting medium (Bioleit; Okenshoji, Tokyo, Japan), and the microscope slides were observed with a conventional and polarized-light microscope (BHS-751P; Olympus, Tokyo, Japan).

For observation of the ultrastructure of xylem parenchyma cell walls, 70-nm-thick sections were cut with a diamond knife, then stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (JEM-100C; JEOL, Tokyo, Japan) at an accelerating voltage of 100 kV (Watanabe et al. 2006).

Confocal laser scanning microscopy (CLSM)—Transverse and tangential sections of xylem (thickness, 20–40 μm) were cut with a sliding microtome. The sections were stained with safranin as described by Kitin et al. (2002, 2003), cleared in xylene and mounted with Bioleit, or left without staining and mounted with glycerol on microscope slides as described by Kitin et al. (2004). Incident-light from an argon ion laser (excitation, 488 nm) and long-pass filter (LP, 590 nm) or a helium neon laser (excitation, 543 nm; LP, 590 nm) were used for observations with a confocal microscope (LSM-310 [Carl Zeiss, Oberkochen, Germany] or C1 [Nikon Optics, Tokyo, Japan]).

Xylem anatomical features—Methods follow those of the IAWA (1989) Committee. Permanent microscope slides from the collection of the Royal Museum for Central Africa in Belgium were used for general description of the xylem anatomy. Tangential diameters of vessel lumina were measured in transverse sections of xylem. In addition, macerated samples of xylem tissue were prepared from small wood blocks soaked in a mixture of equal volumes of glacial acetic acid and 6% hydrogen peroxide and heated at 60°C for 1 or 2 days. The lengths of vessel elements and fibers were measured on macerated samples (Kitin et al. 1999), and the lengths of axial parenchyma strands were measured in tangential sections on digital images that we analyzed with Image J software (available at http://rsb.info.nih.gov/ij; Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA). We compared lengths of vessel elements, fibers, and axial parenchyma strands using independent sample *t* tests.

RESULTS AND DISCUSSION

Xylem structure—Views of transverse, radial, and tangential sections of the wood of *O. aubrevillei* are shown in Figs. 1, 2, and 6. The observed microscopic features according to the IAWA Hardwood Lists (IAWA, 1989) were as follows: diffuse-porous; exclusively solitary vessels with tangential diameters of the lumina in the range of 150–200 μm ($N = 50$), and simple perforation plates; fibers thick-walled with distinctly bordered pits common in both radial and tangential walls (tracheids); rays 3–7 seriate. The horizontal diameters of ray cells in tangential section vary in the range of 20–60 μm ($N = 100$) (Fig. 1C). Axial parenchyma patterns include scanty paratracheal and apotracheal-diffuse and diffuse-in-aggregates (Fig. 1A, 2). Axial strands most commonly contain 2–3 cells, but strands of more than 3 cells occur as well (Fig. 1B, C). The shape of the axial parenchyma cells in tangential sections is frequently irregular rather than oval or tetra-angular (Fig. 1D).

The mean length of fibers was 2010.44 ± 328 μm, which was significantly greater than the mean length of vessel elements (280.69 ± 41 μm; $t = 38.4955$; df = 54; $P < 0.001$), or axial parenchyma strands (291.42 ± 36 μm; $t = 38.3034$; df = 54; $P < 0.001$) (unequal variances were indicated by a preliminary *F*-test). The lengths of vessel elements and axial parenchyma strands are equivalent to the lengths of the initial cambial cells, which also differentiate into fibers (for reviews, see Larson, 1994; Kitin et al., 1999). Hence, it follows that during the xylem ontogeny, the fibers' length in *O. aubrevillei* increases about by a factor of 7 through intrusive growth. A sevenfold elongation ratio of fibers to axial parenchyma during development suggests considerable increase in the mass proportion of fibers relative to that of axial parenchyma.

The morphological definition of fibers with distinctly bordered pits (Baas, 1986; IAWA, 1989) is broader and partly overlaps with the terms true tracheids and fiber-tracheids used

←

Fig. 1. Wood of *Okoubaka aubrevillei*. (A) Bright-field light micrograph (bf) of a general view of transverse section. (B) Longitudinal radial section (bf). C, D, and F. Longitudinal tangential sections (bf). (C) General view. (D) Enlarged view of disjunctive axial parenchyma cells (right) adjacent to a ray (left). Bar = 15 μm. (E) SEM micrograph of a radial section. Tubular protuberances of disjunctive cells pass between fibers. (F) Enlarged view of tubular connections between disjunctive axial parenchyma cells in tangential section (bf). Bar = 20 μm. G, Axial parenchyma cells and fibers in macerated xylem. Arrow points to tubular protuberances of an axial parenchyma cell. Bar = 40 μm. *Abbreviations to all figures:* A, Axial parenchyma; CR, crystals; F, fibers (tracheids); IF, included in rays fibers; R, ray cells; SP, simple pits; T, tubular protuberances of disjunctive parenchyma cells; V, vessel elements.

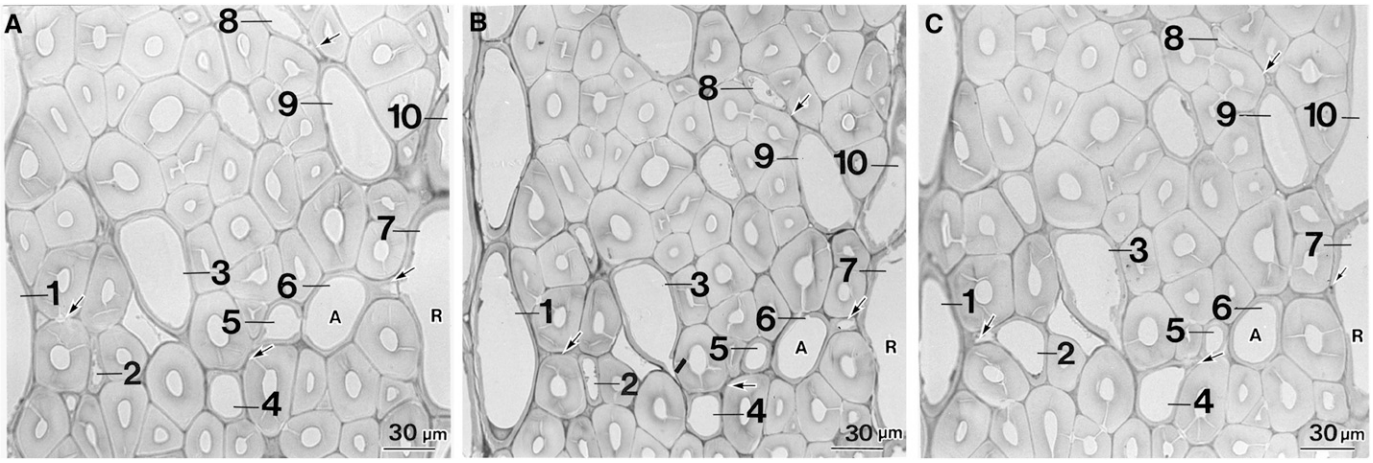

Fig. 2. Selected bright-field light micrographs of a series of cross sections of epoxy embedded wood of *Okoubaka aubrevillei*. The vertical distance between each of the images from (A) through (C) is about 10 µm. Identical disjunctive parenchyma cells are indicated by the same number. Arrows point to the site of tubular connections between disjunctive cells. Note the connections between cells (1 and 2), (3, 4, 5, 6, and 7), and (8, 9, and 10). Although the cells are classified as "diffuse parenchyma" in single sections, along the vertical axis, they are interconnected through tubular protuberances.

by Carlquist (1985, 1986, 2001). Carlquist (1985, 2001) defines the true tracheids as capable of water transport as a subsidiary conducting system when vessels become dysfunctional by embolism, and the fiber-tracheids as more specialized in mechanical function. Sherwin Carlquist (Santa Barbara Botanic Garden, California, personal communication) stated that vasicentric tracheids do occur in the Santalaceae. However, we found a single type of imperforate tracheary elements in *O. aubrevillei*, which are classified as ground tissue true tracheids sensu Carlquist (2001). It has to be noted, however, that the tracheids typically do not elongate much during xylem differentiation and are associated with comparatively long vessel elements (Carlquist, 1975, 1986; IAWA, 1989). In contrast, the imperforate tracheary elements in *O. aubrevillei* are seven times longer than the vessel elements. They still fall in the category "tracheids" by Carlquist (1975, 2001) who considers the nature of the pits and the conductive capabilities as the primary characteristics of defining tracheids, whereas the cells can differ widely in length and wall thickness. The intrusive growth does not prevent formation of bordered pit pairs in the walls of the pointed cell tips (note black arrows in Fig. 6), which supports the assumption for a hydraulic function of these cells.

As discussed earlier, the imperforate tracheary elements in *O. aubrevillei* are thick-walled "fibers with distinctly bordered pits in both tangential and radial walls" (IAWA, 1989) or true tracheids sensu Carlquist (2001). The general term fibers is commonly used for ground tissue, imperforate elements in hardwoods by botanists and foresters. For conciseness, further in the text, we shall call the imperforate tracheary elements in *O. aubrevillei* fibers or thick-walled tracheids.

The majority of axial parenchyma cells appear partly detached from each other with tubular protuberances that connect to neighboring cells (Fig. 1D, F). This architecture is in accordance with Normand (1972), who first defined the xylem parenchyma in this species as disjunctive. The tubular structures of the xylem parenchyma were easily seen in longitudinal sections and in macerated wood (Fig. 1D–G). However, in transverse sections, the connecting tubules between adjacent cells were not always visible because of their positions at various vertical levels (Fig. 2). Three-dimensional analysis of the shape of axial parenchyma cells in a series of transverse sections of the wood blocks better demonstrated the presence of the tubules (Fig. 2A–C). The tubular formations were seen between adjacent fibers (thick-walled tracheids) that separated the disjunctive apotracheal parenchyma cells (see arrows at identical cells in Fig. 2A–C). The tubular formations were also demonstrated in the stereographic images by SEM of longitudinally sectioned wood blocks (Fig. 1E). The orientation of the tubular protuberances was predominantly in the radial direction, and they were easily detected in tangential longitudinal sections under the light microscope (Fig. 1C, D, and F), and by SEM (Fig. 3). However, the protuberances can be oriented in various directions, as seen in series of cross-sections (Fig. 2A–C). The length of the tubules was variable, depending on the cell shape and the distance between the detached cells (Figs. 1F, 3A, B). In some cells, we observed tubular extensions of more than 20 µm long, such as the extensions between cells 3 and 4, and 6 and 7 in Fig. 2A–C. Transverse and longitudinal sections of disjunctive axial parenchyma cells showed that the tubular extensions had thick secondary walls, which were birefringent in polarized light and were similar in thickness to the walls of the body of the same cells (Figs. 1G, 4). Simple to minutely bordered pit pairs with plasmodesmata were present at the ends of the tubular extensions where a tubule of one cell contacted a tubule of another cell (Figs. 3C, D, 4B). In addition, no apparent difference between the axial and ray parenchyma cells was detected regarding the structure of pits. In the walls of both types of cell, the pits had diameters of 3–6 µm, and plasmodesmatal channels were present in the pit membranes (Fig. 5). The plasmodesmatal interconnection between adjacent living parenchyma cells is a consistent feature of the secondary xylem (reviewed in Barnett, 2006). In addition, bordered pits commonly occur in thick lignified walls of ray and axial parenchyma in many species of woody dicotyledons, which may be related to mechanical strength of the parenchyma wall (Carlquist, 2007).

In other tree species, disjunctive cells have been found predominantly in rays and rarely in axial parenchyma (IAWA, 1989; Carlquist, 2001). However, in *O. aubrevillei* disjunctive cells of the rays were not apparent and were observed

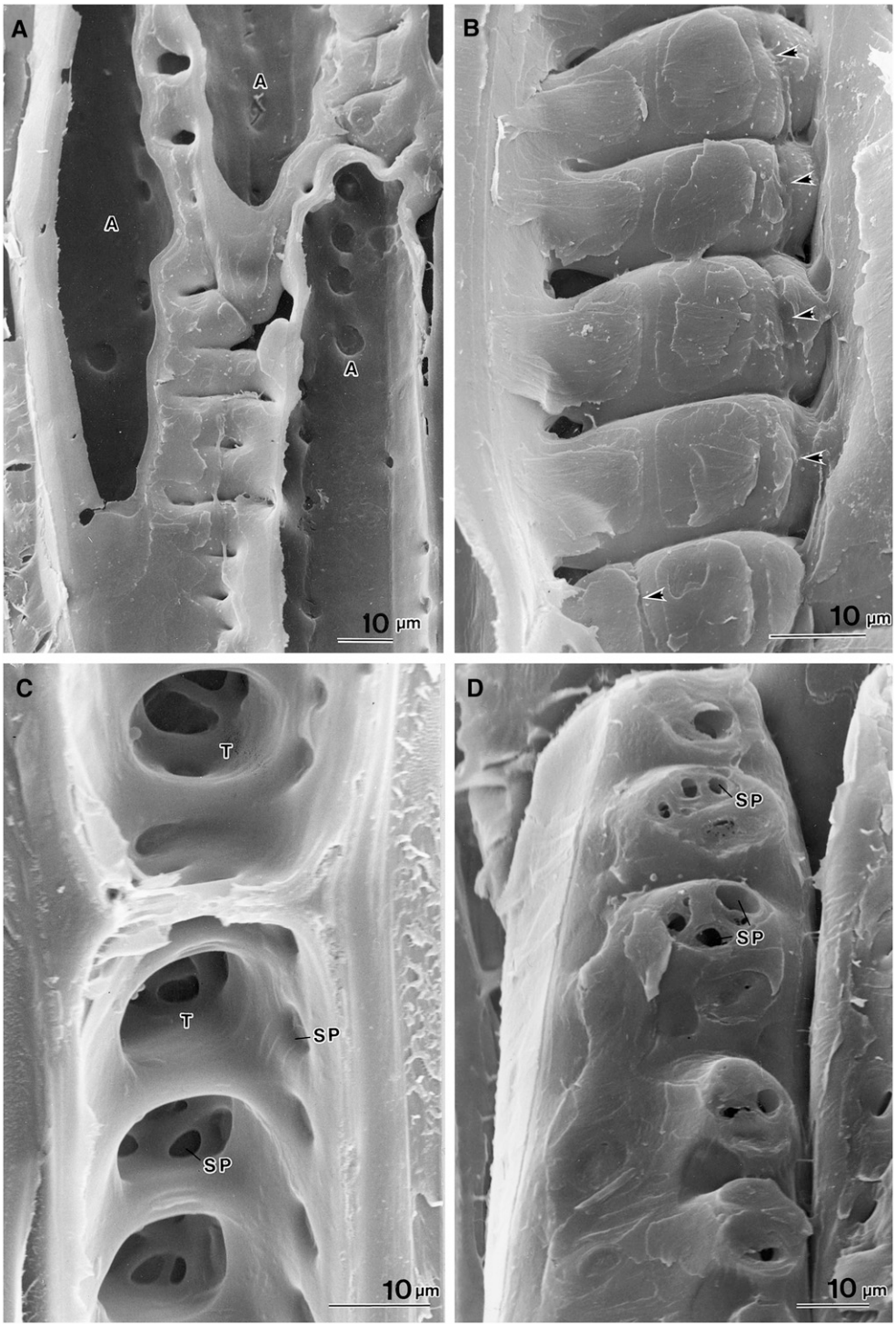

Fig. 3. SEM micrographs of xylem of *Okoubaka aubrevillei*. (A) Tangential view of longitudinally sectioned disjunctive axial parenchyma cells. (B) Tubular protuberances of disjunctive axial parenchyma cells. Arrowheads point to the connection points between adjacent cells. (C) View from the lumen side of disjunctive parenchyma cells. Tubular protuberances with pits in the walls are seen from the lumen side. (D) View from the outer side of a disjunctive parenchyma cell. Tubular protuberances with pits at their tips are seen from the outside. The pit membranes are partly removed during sectioning.

only in a few sections. Such cells frequently occurred between axial and ray parenchyma cells (Figs. 2 and 5B) and among the axial apotracheal and scanty paratracheal parenchyma (Figs. 2 and 6).

***Pattern of formation of disjunctive parenchyma cells*: *The* "*mechanistic*" *hypothesis*—**A "mechanistic" hypothesis has been proposed to explain the unusual appearance and origin of the disjunctive cells in xylem (Esau, 1965a, see p. 47; Normand,

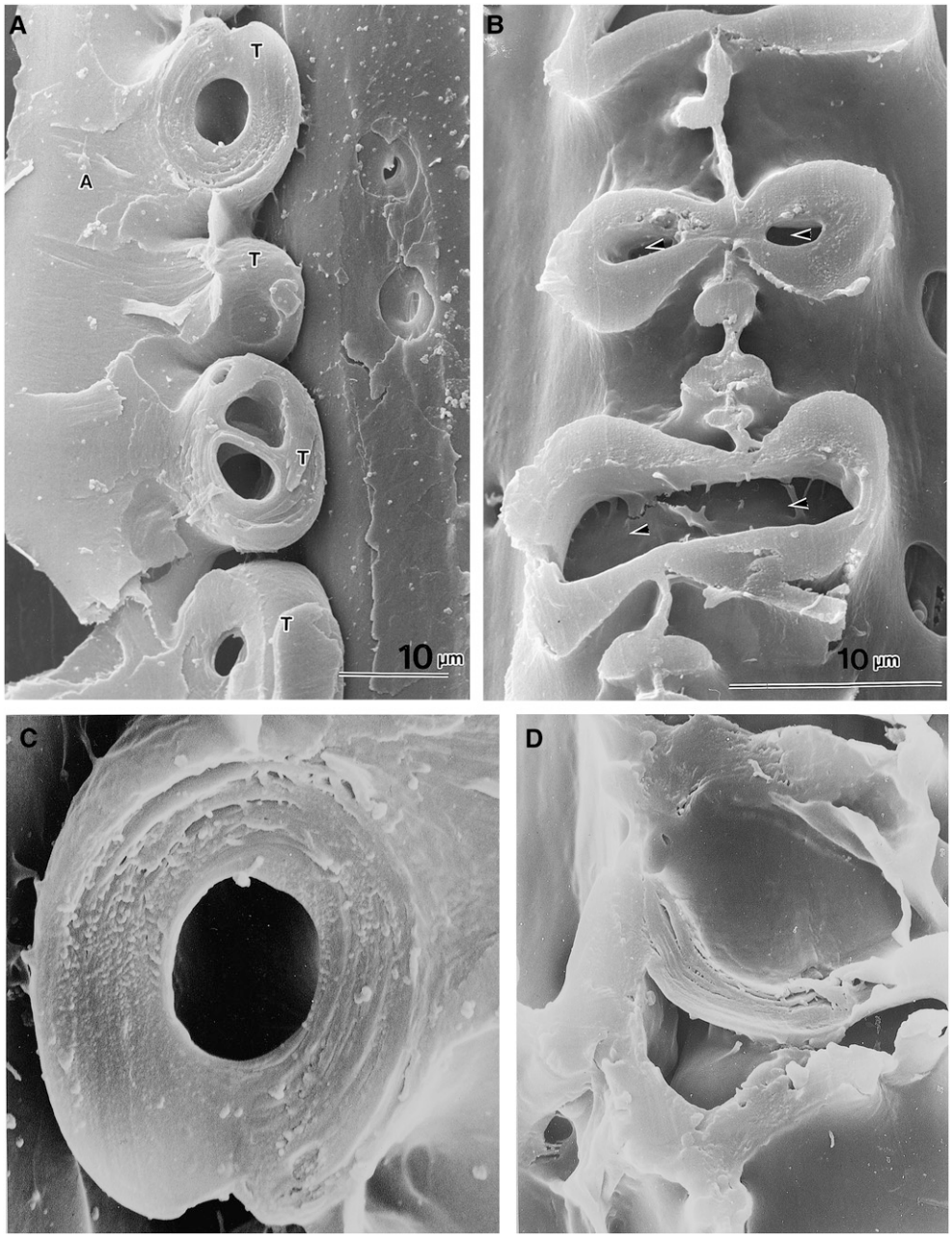

Fig. 4. SEM micrographs of longitudinal sections of xylem showing disjunctive axial parenchyma cells of *Okoubaka aubrevillei*. (A) Cross section through tubular protuberances. (B) Longitudinal section (LS) through tubular protuberances of adjacent disjunctive axial parenchyma cells. Simple or slightly bordered pits are seen at the contact points of the tubules of adjacent cells. Arrowheads point to intercellular spaces. (C) Enlarged view of the upper tubule in (A). (D) LS through tubular protuberance of disjunctive axial parenchyma cell. The secondary wall appears to have a multilayered structure (see also C). Scale bar in (B).

1972; Metcalfe and Chalk, 1989). Disjunctive wood elements have been assumed to form as a result of mechanical tensions and rearrangements of the wood tissue due to different rates of growth of various xylem cells during the process of xylem differentiation. For example, some vasicentric tracheids or parenchyma cells have been assumed to become disjunctive through the pressure of the expanding vessel elements forcing adjacent cells apart. However, Fujii et al. (1994) found in the *Sophora* group (Fabaceae) that disjunctive axial parenchyma cells were not always associated with vessel elements and that such cells can also occur in species with vessel elements of less than 100 μm in diameter. Therefore, they suggested that formation of disjunctive cell walls of axial parenchyma cells may be induced mainly by the intrusive growth of fibers. Such a mechanism could also be responsible for the formation of disjunctive cell walls of the apotracheal parenchyma in *O. aubrevillei*, in that the disjunctive parenchyma of this species is mostly within the ground tissue of fibers (thick-walled tracheids), which are seven times longer than the axial parenchyma strands/vessel elements. In other species, such as sessile oak (*Quercus petraea*), which

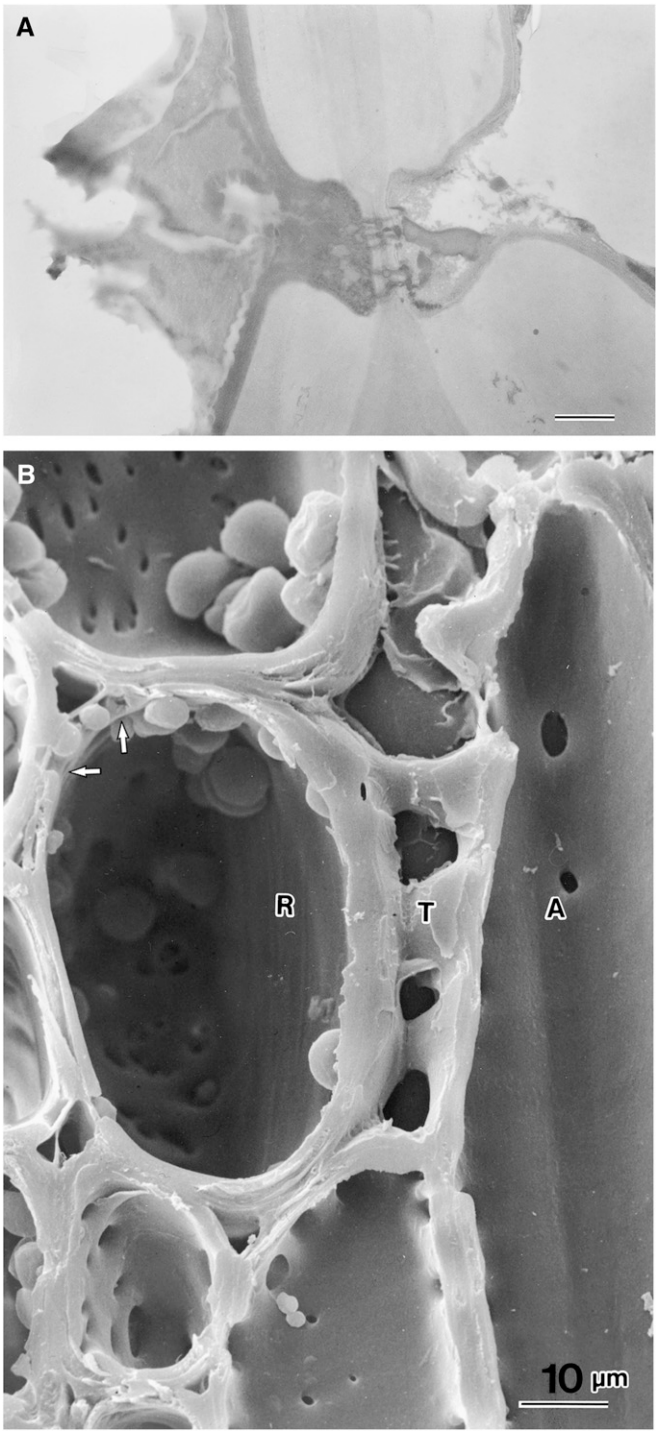

Fig. 5. Longitudinally sectioned xylem of *Okoubaka aubrevillei*. (A) TEM micrograph showing a pit pair in the adjacent cell walls of xylem parenchyma cells. Plasmodesmata are seen in the pit membrane. Bar = 1 µm. (B) SEM micrograph showing a tangential view of disjunctive ray and axial xylem parenchyma cells. Horizontal arrow points to a pit pair between ray cells, and vertical arrow points to a blind pit to intercellular space.

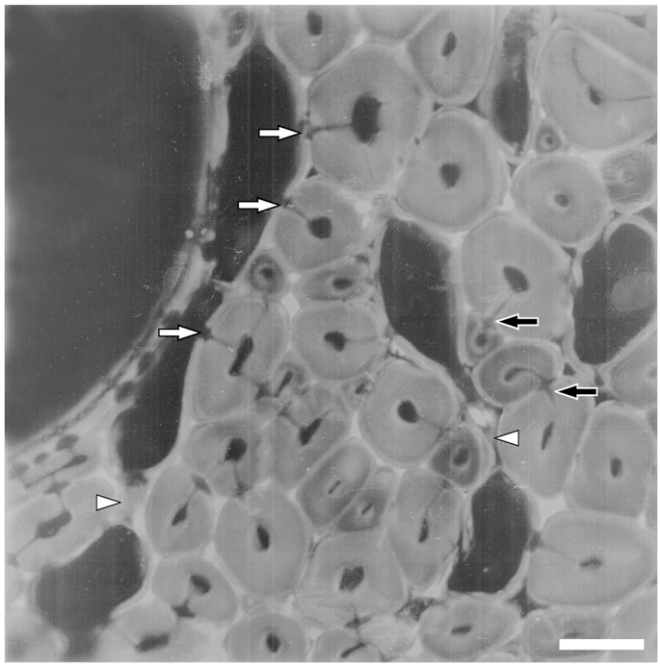

Fig. 6. Transverse section of wood of *Okoubaka aubrevillei*, ~40 µm thick, stained with safranin. Confocal microscopy image viewed with excitation by a helium neon laser (543 nm) and a long-pass filter (590 nm). Arrowheads point to tubular protuberances of axial parenchyma cells. White arrows point to pits between axial parenchyma cells and adjacent fibers (tracheids). Black arrows point to pit pairs connecting the intrusively grown tips of fibers (cells with small diameters) with the central portion of adjacent fibers (cells with larger diameters). Such pit connections have been additionally established between adjacent fibers during the differentiation of xylem. Scale bar = 30 µm.

lacks disjunctive apotracheal parenchyma, fibers are only 2–3 times longer than vessel elements (Heli ska-Raczkowska and Fabisiak, 1991). The mostly apotracheal arrangement of axial parenchyma and the considerable elongation rate of fibers suggest that the separation of adjacent parenchyma cell walls could result from an intensive intrusive growth of differentiating fibers. In differentiating xylem, the middle lamella of axial parenchyma cells is not lignified and is probably not resistant to tensile forces. Mechanical forces induced by the intrusively growing tips of elongating fibers could cause separation of the middle lamella and the disjunction of adjacent parenchyma cells. However, by some mechanism, owing to the extensibility of plasmalemma and cytoplasm, the plasmodesmata between adjacent cells are not disconnected, and the complete separation of disjunctive cell walls is prevented, sustaining the continuum of the symplast.

Xylem parenchyma cells form secondary walls later than their neighboring vessel elements (Kitin et al. 2003). Because of this delay in differentiation, the primary walls of parenchyma cells could possibly be extended at the cell-to-cell symplast communication points that form the tubule-like structures. The deposition of a thick secondary wall in the tubular extensions, such as that seen in Fig. 4, could only occur after the elongation of fibers has ceased.

Perspectives for further research on the mechanism of disjunctive cell wall development—Our results illustrated the three-dimensional shape of disjunctive cells and strongly supported the hypothesis for a cell–cell separation mechanism leading to disjunctive parenchyma. However, the mechanistic hypothesis does not entirely explain the formation of disjunctive

cell walls, and several questions regarding the development of tubular contacts are still unanswered. That only certain portions of the double cell walls of xylem parenchyma can be separated might be related to a heterogeneity in the structure of primary walls. Several studies of different species have shown that the cell wall is thinner at the primary pit fields, and the orientation of microtubules at the pit membranes differs from the adjacent primary wall areas (Barnett and Harris, 1975; Abe et al., 1997; Funada et al., 2001; Kitin et al., 2001). Primary pit fields, which are rich in plasmodesmata and distinct in structure, may behave differently than other areas of the cell wall. Earlier studies by Wenham and Cusick (1975), Barnett and Harris (1975), Barnett (1981), and Catesson et al. (1994) have shown dynamic changes in the structure of the middle lamella during differentiation of xylem cells. Those earlier studies on xylem formation suggest that investigating the ultrastructure of the middle lamella and the plasmodesmata-containing primary walls of developing parenchyma cells in *O. aubrevillei* could elucidate the mechanism that allows symplast extension and prevents the complete separation of cells. Furthermore, the xylem with disjunctive parenchyma may be an interesting model for studying the mechanisms of growth of xylem cells and the plasticity of the primary wall.

Whereas dried wood samples were sufficient for our purpose of studying the morphology of disjunctive xylem parenchyma, the detailed understanding of the process of disjunctive wall formation would require the study of differentiating xylem.

What are the consequences of the structural difference between wood with and without disjunctive parenchyma?—The mechanistic hypothesis explains the origin of disjunctive parenchyma as being the result of mechanical forces of cell readjustments during xylem development. In addition to the mechanics of wood formation there could be a physiological strategy of the species to develop disjunctive parenchyma.

A systematic investigation of the wood structure of Central African trees found that disjunctive xylem parenchyma is common in many tropical species and different taxonomic groups (H. Beeckman and P. Kitin, unpublished results). It is not known why disjunctive xylem parenchyma is more frequent (or better developed) in the tropics than in the temperate zones. Similarly, xylem parenchyma in general tends to be more abundant in tropical than in temperate species, but the reason is unclear (Baas, 1982; Wheeler and Baas, 1991; Alves and Angyalossy-Alfonso, 2002). While this is still an open discussion, a higher proportion of parenchyma probably points toward the evolution to higher complexity and more pronounced specialization in tropical woods. Many of the resource levels in the tropical rainforests are constantly abundant (high temperatures, high precipitation). There is a year-round input of high quality energy from the sun. Severe interspecies competition also enhances the level of complexity; the number of species and their internal organization are much higher. Much more variation in growth forms and xylem structure is seen in the tropics: a higher proportion and diversity of parenchyma, disjunctive parenchyma, secretory tissues, septate fibers, larger differences in the lengths between vessel elements and fibers, and sometimes "anomalous" xylem structure such as included phloem. Most of these interesting phenomena in tropical woods still need ecophysiological explanations.

The 3D architecture of the disjunctive parenchyma in *O. aubrevillei* shows that these types of cells combine features of the parenchyma defined as "diffuse" and defined as "apotracheal in narrow tangential bands" (IAWA, 1989). Diffuse and diffuse-in-aggregates are transitional arrangements because, as discussed by Carlquist (2001, p. 161), "where diffuse axial parenchyma cells are more abundant, random distributions of axial parenchyma cells inevitably result in small groupings here and there." According to the classification scheme of Kribs (1937), the diffuse axial parenchyma is the most primitive type of arrangement and is associated with longer vessel elements. The "aggregated" type of parenchyma is usually present in more advanced woods, in combination with other specialized wood elements. Although, specialization trends and phylogeny of different types of parenchyma can be complex and disputable (Baas, 1982; see also Metcalfe and Chalk, 1989, p. 117), aggregated types of parenchyma are commonly considered to perform more efficiently their storing and conductive functions by better "bridging" between axial and radial parenchyma systems (Carlquist, 2001). As shown in the scheme in Fig. 7, diffuse parenchyma composed of disjunctive cells provides good bridging between the axial and radial systems of parenchyma and also can be considered efficient for conductive function.

According to Carlquist (2001), diffuse parenchyma often occurs in woods with tracheids and may represent a way of dispersing parenchyma among water-conducting cells. Similarly, the fibrous ground tissue of *O. aubrevillei* is composed of thick-walled tracheids (fibers with distinctly bordered pits in both radial and tangential walls), and pit contacts occur frequently between tracheids and disjunctive axial parenchyma (white arrows in Fig. 6). Such pit pairs between axial parenchyma and fibers (thick-walled tracheids) may have a hydraulic function, but we know little about this yet.

The phylogeny of xylem with disjunctive parenchyma might indicate parallel evolution of the fibrous and parenchymatic xylem tissues. A higher rate of xylem fiber elongation leads to a greater proportion of specialized mechanical elements, which, in a species such as *O. aubrevillei*, are accompanied by disjunctive

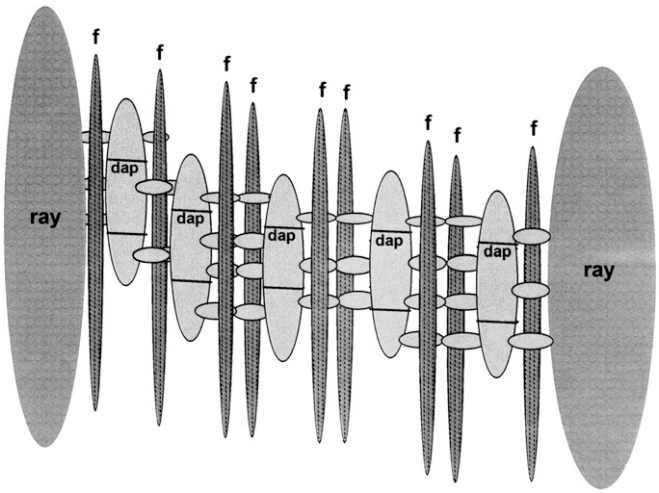

Fig. 7. Schematic representation of the network of parenchyma cells in wood of *Okoubaka aubrevillei*. The disjunctive axial parenchyma cells (dap) are connected through tubular protuberances with each other and with the parenchyma of rays (ray). The tubular protuberances pass between intrusively elongated fibers (f). Each living parenchyma cell (dap and ray) is part of a common symplast of the xylem. Note that in reality the mean length of fibers is about seven times greater than that of axial parenchyma strands. Also for simplicity, vessels are not represented in the scheme.

parenchyma. The resulting structure may promote the mechanical and the conductive (or storing) functions of wood: the proportion of thick-walled tracheids (mechanical and water conductive/storing function) is increased without compromising the functionality of parenchyma because the number of cells and the connection between living protoplasts are maintained through tubular protuberances. The multilayered and lignified walls with minutely bordered pits of the parenchyma cells may additionally contribute to the mechanical strength of the wood. Maintaining the connections of parenchyma cells into a dense network may have a damper (shock-absorber) effect on mechanical stresses in analogy to what has been reported about the performance of bamboo ground parenchyma (Obataya et al., 2007).

A variety of biomechanical designs of xylem structure corresponding to diverse habitats coexist in nature (reviews in Gartner, 2001; Woodrum et al., 2003; Rowe and Speck, 2005; Read and Stokes, 2006). As mentioned earlier, *O. aubrevillei* is a large, fast-growing tree, with a comparatively high wood specific gravity. Its xylem structure, with a high elongation rate of fibers (thick-walled tracheids) and the formation of disjunctive parenchyma, could be an interesting example of how plants deal with the tradeoffs between mechanical and conductive/storing functions of xylem.

This three-dimensional study of the shape and spatial arrangement of disjunctive xylem parenchyma in *O. aubrevillei* provides new insights on the variation and complexity of xylem structure. However, interesting questions on the mechanisms of formation of disjunctive cells as well as on their role in the xylem function remain to be answered. Further research on the differentiation process and the biomechanics of wood with disjunctive parenchyma is desirable for further understanding of the disjunctive xylem cell phenomenon and its biological significance.

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