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ORIGINAL ARTICLE

Cambial activity in the understory of the Mayombe forest, DR Congo

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Abstract

Key message **Understanding species-specific response as well as wedging and zero xylem growth is vital for tree-ring analysis of tropical understory trees.**

Abstract Knowledge on intra-annual xylem growth remains understudied in tropical regions, especially for understory species. However, it is important to disentangle seasonal tree response in this complex environment. The aim is to assess intra-annual wood formation and its variability in selected understory tree species of a semi-deciduous tropical forest. The cambium of four species from the Luki reserve of the Mayombe (DR Congo) was monthly marked at the stem base via the pinning method. To assess ring anomalies on the stem disks, digitization of the last 5–10 rings was performed along the circumference. Relative growth was determined based on X-ray CT volumes of the pinning zone, as well as on sanded surfaces and microsections. Stem disks allowed to visualize ring anomalies and growth variations. Intra-annual growth was successfully derived via X-ray CT and could be fitted with a Gompertz function. A species-specific response is observed, although there is circumferential variability. However, the most remarkable result is that many of the trees in the data set had no xylem formation at the stem base, throughout the entire season, thus forming missing rings. Intra-annual variability in growth illustrates the different responses of species and individual trees to environmental drivers. Phenology might explain the differences, although site and competition should be considered as well. A large number of trees show no xylem growth at all, apart from wound-induced local growth, causing missing rings which have important implications for the tree-ring analysis in tropical regions.

Keywords Cambial pinning · Mayombe · Tropical tree growth · Tree-ring analysis · X-ray CT · Zero growth · DR Congo

Introduction

Studying tree rings in Africa is vital for the assessment of tree response to a changing climate (Gebrekirstos et al. 2014). Due to lower seasonal changes in tropical regions, tree-ring analysis is often difficult to perform and more information is needed to assess the climate response. Adding intra-annual growth information to these rings leads to specific information that increases our knowledge on how tropical trees react on, for instance, extreme events, such as drought, occurring occasionally within the growth season. Such droughts can lead to structural changes within the tree-ring boundaries, causing intra-annual density fluctuations (Battipaglia et al. 2010). Despite the importance of revealing intra-annual response of tropical trees, only few studies exist on intra-annual xylem formation (Bauch et al. 2006; Marcati 2006; Trouet et al. 2012), and, especially, in

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the wetter tropics (Amobi 1973; Dié et al. 2012; Morel et al. 2015). Intra-annual wood growth has extensively been studied on conifers (Camarero et al. 2010; Rathgeber et al. 2011; Cuny et al. 2015) as well as on angiosperm trees (van der Werf et al. 2007; Michelot et al. 2012), through various techniques, such as dendrometers (Deslauriers et al. 2003), micro sampling (Rossi et al. 2006, 2013), and cambial pinning, each with their advantages and disadvantages (Mäkinen et al. 2008). Low cost, ease of implementation and the possibility of calculating relative growth (Seo et al. 2007) promote the use of cambial pinnings, especially in remote regions. The cambial pinning technique, first introduced by Wolter (1968), is used to periodically wound the cambium, to derive intra-annual wood formation. A thin needle is inserted through the bark and cambium into the xylem of the tree. The wounding initiates the formation of wound tissue and modified wood reaction cells around the pinning canal (Couralet 2010). The wound reaction can be used as a timer to monitor the amount of wood formation within a certain time period (Seo et al. 2007). Many studies involving pinnings in the tropics are often merely used to check whether rings are formed annually (Mariaux 1967; Dünisch et al. 2002; Verheyden et al. 2004; Lisi et al. 2008; Chowdhury et al. 2015; Remane and Therrell 2015), thus not revealing the total intra-annual information within the tree ring. Therefore, intra-annual studies are urgently needed.

However, several challenges occur in revealing the tropical tree response: given the intrinsic plastic behaviour of the cambium in trees growing in regions with less pronounced seasonality (Cherubini et al. 2013), wedging rings and false rings occur and they are mostly described through the tree-ring analysis as a cross-dating problem (Worbes 2002). To our knowledge, this circumferential variability is not being considered during cambial pinning experiments (but see Krepkowski et al. 2012 and Lupi et al. 2013 for micro-coring), but especially relevant in the tropics. It is also a tedious procedure to prepare proper samples (Mäkinen et al. 2008) and tropical hardwoods have a more complex anatomy compared with conifers, which requires methodological improvements in some cases. Moreover, specifically, the evergreen understory and canopy species still remain largely understudied in Africa.

This paper explores intra-annual xylem formation for selected canopy and understory trees of the Luki Biosphere Reserve. We hereby complement the previous research in the study area (Couralet et al. 2010), which has shown species-specific response based on dendrochronology and seasonal diameter measurements. X-ray Computed Tomography (X-ray CT) imaging was proposed as a method to infer intra-annual xylem growth; it has been shown before that X-ray CT can be used to assess the wound tissue non-destructively in 3D (Stoffel and Klinkmüller 2013; Ballesteros-Cánovas et al. 2015). In this study, the primary objective was to analyse the variability of intra-annual xylem formation between species, between individuals and finally at the individual level.

Materials and methods

Study site

The Luki Biosphere Reserve is located in the west of the DR Congo, 120 km from the Atlantic Coast and the most southern part of the Mayombe forest (Fig. 1), and may be considered as representative for the flora of the Mayombe (Lubini 1997; Couralet 2010).

The Luki reserve can be classified as tropical semi-evergreen forest of the Guineo-Congolean forest domain, and consists of a mixture of evergreen and deciduous species in the upper-stratum and mostly evergreen species in the understory (Lubini 1997). The soils are generally determined as ferralitic, acid, and with poor chemical content (Senechal et al. 1989). The region is assigned to tropical savanna climate or tropical wet and dry climate, corresponding to the 'Aw' climate of the Köppen climate classification (Peel et al. 2007), lies at the edge of the Atlantic Equatorial Coastal forest ecoregion (Olson et al. 2001) and the forest type is classified as dry forest and thicket (White 1983). This makes the Luki reserve an important location concerning tree response on environmental forcing. June to September, with a monthly precipitation less than 50 mm, defines a distinct dry period. In general, these low precipitation values would not be favorable for the presence of the dense humid Mayombe forest (Couralet 2010). However, the cold Benguela stream in the gulf of Guinea creates a thick, low-level, non-precipitating cloud layer during the dry season (Pendje and Baya 1992). During this dry period, the inter-tropical convergence zone moves north, yet a thick cloud cover results in high relative humidity as well as a decrease in temperature, due to a lower sea temperature. As a consequence, the solar irradiance and temperature are lower than average, yet relative air humidity remains high all year long. Plants, most probably, do not experience extreme water stress during the dry period (Lubini 1997; Couralet et al. 2010).

The selected individuals all grew in the same old-growth forest stand, close to the INERA (Institut National pour l'Etude et la Recherche Agronomiques) research station of Luki. Species were selected based on their abundancy, ability to form growth rings (Fouarge and Gérard 1964; Couralet et al. 2010b), and degree of wedging, based on the previous sample campaigns (data not shown). Selection criteria in the field were health, maturity, and, if possible, roundness and straightness of the stem.

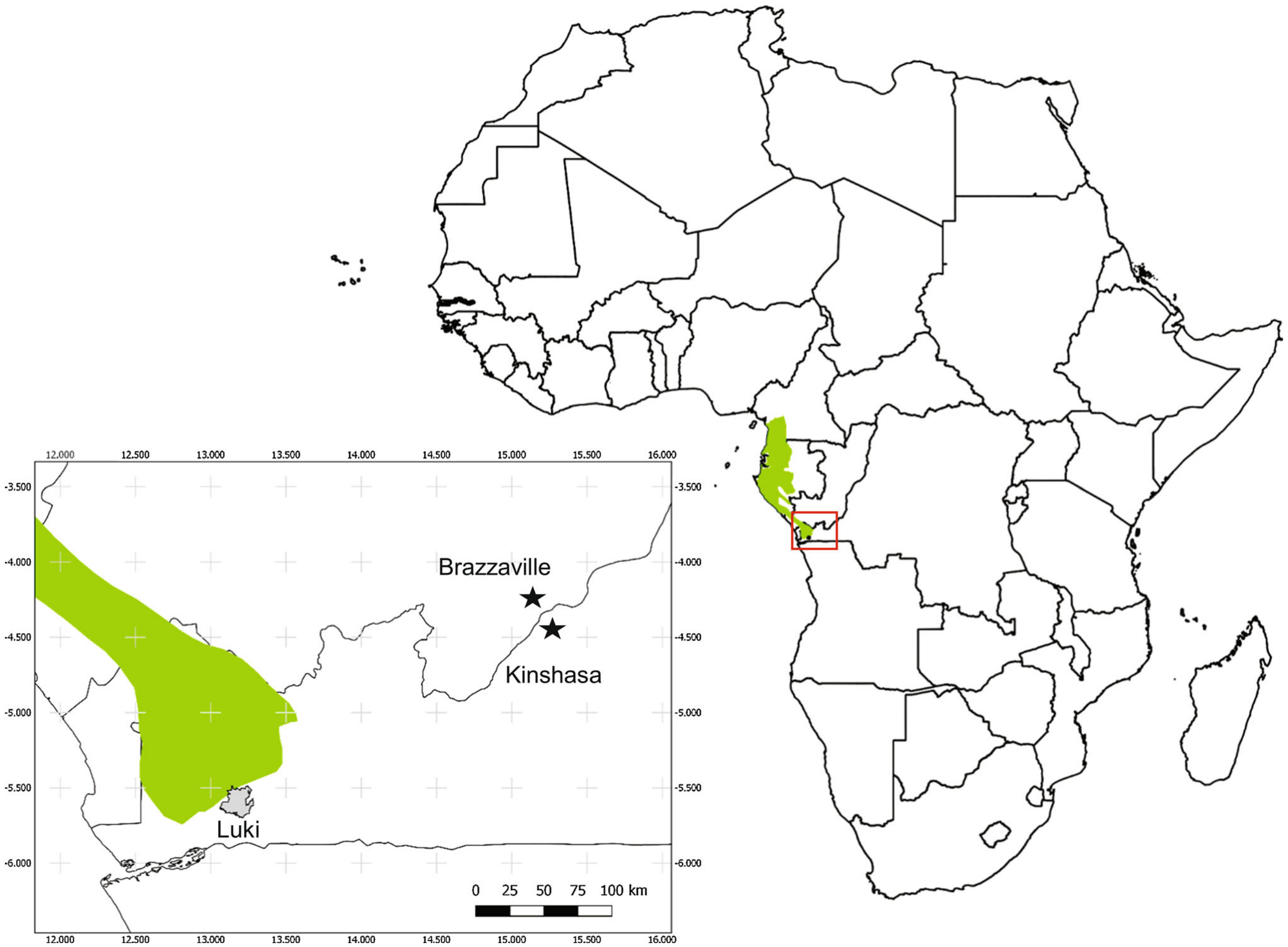


Fig. 1 Location of the Luki Biosphere Reserve in the DR Congo, at the edge of the Mayombe. The depicted Atlantic Equatorial Coastal forest ecoregion delimitation is adopted from Olson et al. (2001)

Cambial marking and sample preparation

Twelve trees of four different species (3 *Greenwayodendron suaveolens*, 3 *Xylopia wilwerthii*, 3 *Corynanthe paniculata* and 3 *Aidia ochroleuca*) in the same site were selected and pinned monthly with a straight pin during one growth season (11 months, starting from 21/09/2013 till 21/07/2014). Each monthly pinning was repeated three times in a spiral arrangement (Fig. 2), shifted 5 cm to one another to avoid interference of nearby pinnings. The selected species are evergreen mesophanerophytes (8–30 m in height), except for *G. suaveolens,* which is a megaphanerophyt (more than 30 m in height). All species are widespread in the Guineo-Congolean forest domain.

In September 2014, all trees were felled and stem disks, including the pinnings, were sampled. Subsequently, stem disks were dried at room temperature (about 25 °C) during a period of 4 months after which disks were subdivided into three smaller disks each containing 11 consecutive monthly pinnings. In addition, pinning samples for *A. ochroleuca*, *C. paniculata*, and *X. wilwerthii* were available from a previous pinning campaign in the season 2006–2007, from a site nearby the same study area (Couralet et al. 2010).

Flatbed imaging of stem disks and X-ray computed tomography of pinnings

The stem disk surface was progressively sanded (50–600 grid size) for proper visualization of the macroscopic tree-ring structure. The pinned stem disks were scanned with a flatbed scanner (EPSON Perfection 4990 PHOTO), at a resolution of 1200 dpi, followed by the digitization of the last 5–10 rings with the ImageJ (Schneider et al. 2012) to identify ring wedging. These rings can serve as an overlay and enable the detection of anomalies before sampling of the pinnings for further analysis.

Cylinders of approximately 1.5 cm in diameter and 1 cm in length containing the pinnings were then sampled from the stem disks. These cylinders were scanned with X-ray CT, resulting in a 3D view on the wood structure

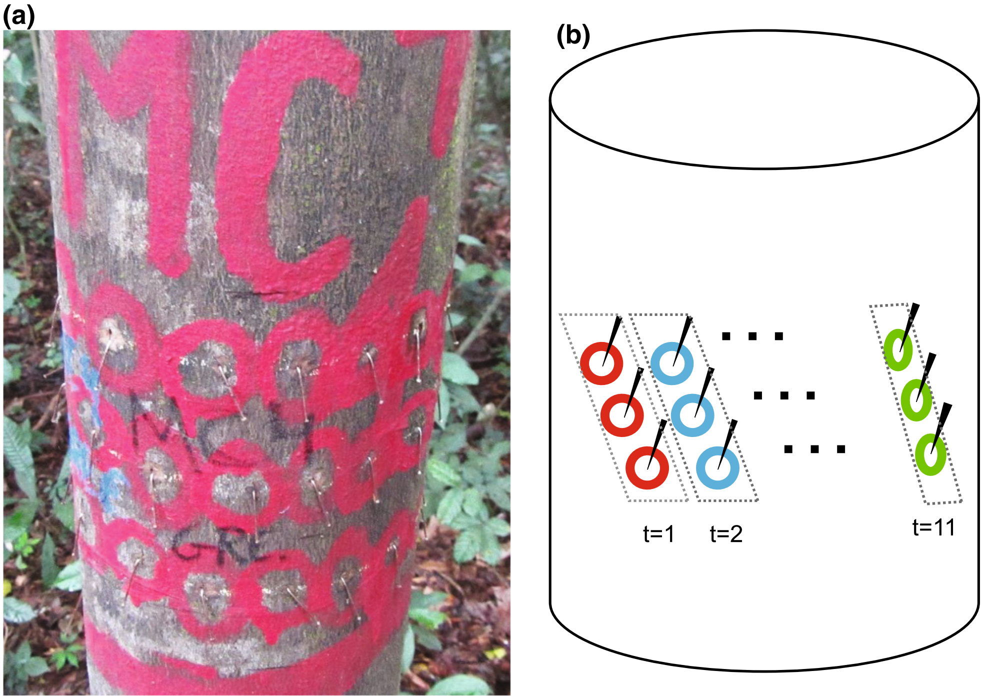


Fig. 2 *G. suaveolens* individual with **a** monthly cambial pinnings and **b** corresponding sample scheme of pinnings and replicates

around the pinned zone. This allowed to assess the pinning structure and quality before sanding or cross sectioning.

The Nanowood scanner used at Woodlab-UGent is a setup developed at UGCT (Dierick et al. 2014), the Ghent University Centre for X-ray Tomography, in collaboration with XRE (http://www.XRE.be). Specimens were scanned with the standard cone-beam X-ray CT with a scan time of approximately 21 min per sample. Reconstruction was performed with Octopus, a tomography reconstruction package for parallel, cone-beam geometry, and helical geometry, as well as phase correction and retrieval (Vlassenbroeck et al. 2007, licensed by InsideMatters: http://www.insidematters.eu). Beam-hardening correction was applied, both by hardware as well as software filtering. The obtained approximate voxel pitch is 10 µm.

Anatomical differences between regular wood structures and wound-induced features enable the localization of the cell boundary before and after pinning of the cambium. X-ray CT guided thin cross sections of pinning sections were taken for detailed study of the wound tissue if relative growth could not be measured on the X-ray CT volumes.

Intra-annual xylem formation

The increment from the beginning of the growth season up to the pinning date was measured. Following Seo et al. (2007), the increment up to the harvesting date was additionally measured to correct for eccentric xylem growth. The measurements provide a time series of relative radial growth during almost one growth season.

A Gompertz function (Gompertz 1825 in Winsor 1932) was fitted to the time series of relative growth data. The Gompertz function has been applied in several studies of radial xylem growth in boreal and temperate regions (Deslauriers et al. 2003; Rossi et al. 2003; Mäkinen et al. 2008), but applications for tropical regions are not reported. Because of its flexibility and asymmetrical shape, the Gompertz equation has been proven successful to describe growth–time relationships (Zeide 1993; Mäkinen et al. 2008). The Gompertz equation is defined as:

$$\mathrm{RG} = ae^{-e^{\beta-\kappa t}}$$

where RG is relative growth, a is the upper asymptote, β is the x-axis placement for the location of the origin (Cuny et al. 2013), κ stands for the rate of change parameter, and t is the time expressed as the number of days from the first cambial pinning (here, 21/09/2013 and 01/10/2006 for the considered seasons). All processing steps were done in Matlab R2015b®.

The cylinder samples were cut to cubes of about 1 cm^3 containing the pinning. These cubes were heated in the oven for 24–48 h at 70 °C in a mixture of 60 % H_2O, 30 % glycerol, and 10 % alcohol to soften the tissue for proper micro-sectioning. Transversal sections with a thickness of 20–30 µm were cut on a sliding microtome and coloured with safranin/astra blue and fixed in Euparal resin.

Results

An overview of the sampled trees with their biometric parameters is given in Table 1. Some pinnings could not be used, because the needle did not reach the cambium due to the thick bark.

Digitized ring contours and X-ray CT-based relative growth measurements

The digitization of stem disks and ring indications allows mapping of irregular stem growth and assessing the impact of wedging (Fig. 4). Thus, wedging rings and eccentric growth can easily be detected. Furthermore, this allows to locate the pinnings accurately on the digitized disks when subsampled for X-ray CT.

Using X-ray CT imaging, one is able to section the pinning virtually, at any direction to search for the best cross section to perform the measurements (Fig. 3). The axial region to perform the distance measurement is not always right on the pinning spot, but rather more above and below the wounding. Bright spots around the pinning zone are due to iron oxide of the needle as well as wound-induced depositions. Xylem growth is visible after the wound formation, thus distances can easily be measured (arrows Fig. 4a).

Table 1 Overview of the studied samples

Season	Species	No. of trees	Tree height range (m)	Dbh range (cm)	Digitized disks	X-ray imaging	Intra-annual curve	Zero growth
2013–2014	*Greenwayodendron suaveolens*	3	20–25	22.9–34.5	3	3	3	–
	Aidia ochroleuca	3	12–14	22.6–26.3	3	3	3	–
	Corynanthe paniculata	3	12–22	12.4–27.5	3	3	1	2
	Xylopia wilwerthii	3	11–12	18–29.3	3	3	3	–
2006–2007	*Aidia ochroleuca*	4	–	14.9–23.0	–	–	4	–
	Corynanthe paniculata	7	–	18.4–46.0	–	–	5	2
	Xylopia wilwerthii	6	–	15.4–21.0	–	–	–	6

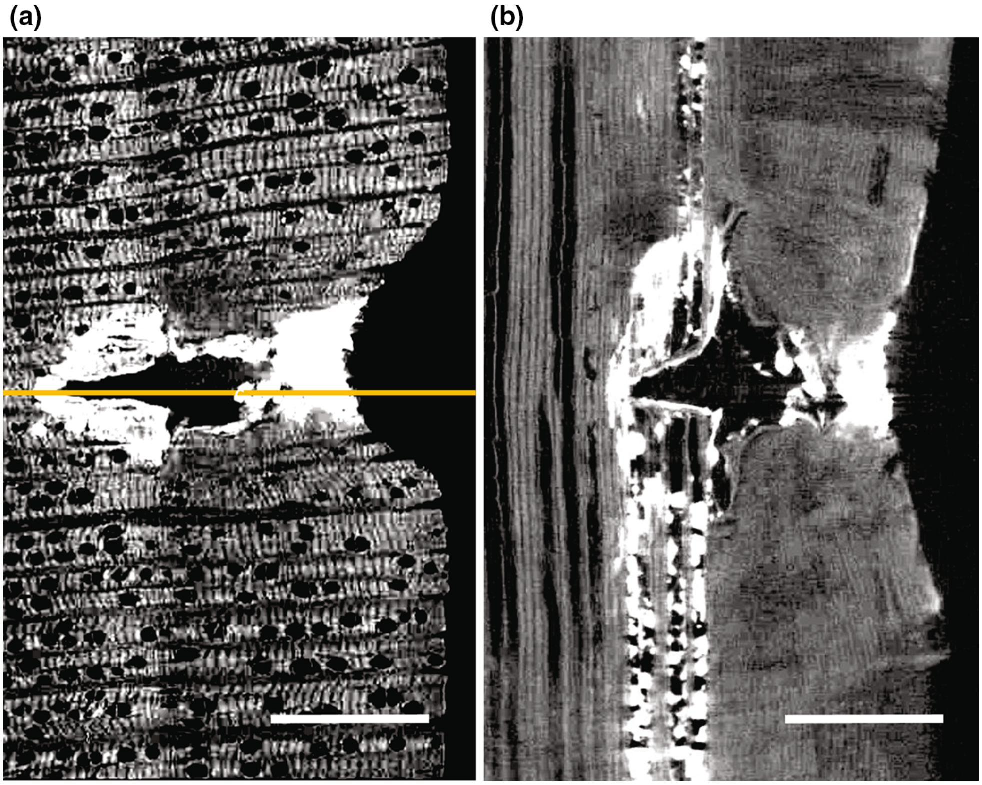
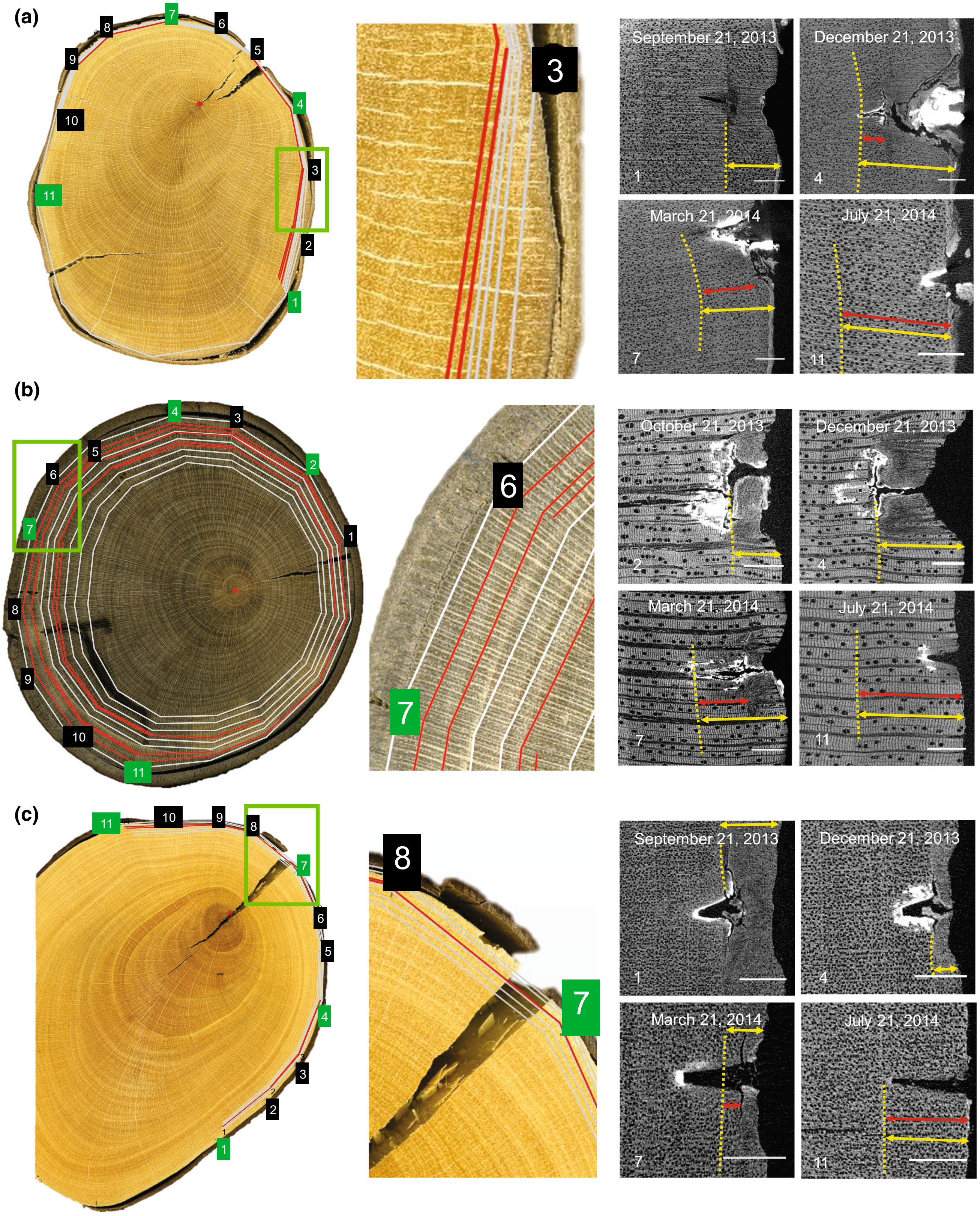


Fig. 3 X-ray CT images allow to sample the best cross section for relative growth measurements. **a** Transversal view with a section (*horizontal line*) of the **b** radial view. *Scale bar* 1 cm


(a)
8
7
6
9
5
4
10
3
11
2
1
3
September 21, 2013
1
December 21, 2013
4
March 21, 2014
7
July 21, 2014
11
(b)
4
3
5
2
6
7
1
8
9
10
11
6
7
October 21, 2013
2
December 21, 2013
4
March 21, 2014
7
July 21, 2014
11
(c)
11
10
9
8
7
6
5
4
3
2
1
8
7
September 21, 2013
1
December 21, 2013
4
March 21, 2014
7
July 21, 2014
11

◀**Fig. 4** Stem disk images with digitized contours of the last 5–10 rings, including wedging rings, derived from digitized stem disks for **a** *A. ochroleuca*, **b** *G. suaveolens,* and **c** *C. paniculata*. *White lines* represent normal rings; *red lines* indicate wedging rings. For **a** and **c,** measurements were only performed on a section of the total circumference. Indication of the measurements on the X-ray cross section is shown: *dashed line* is the tree-ring boundary, *red arrows* indicate the growth till pinning, *yellow* the total ring width to achieve relative growth. *Scale bar* 1 mm

Species-specific xylem formation

All species in this study show xylem growth cessation during the dry season: on the X-ray CT pinning images of July, only the needle insertion is visible and the tree ring has fully developed (Fig. 3). A species-specific xylem growth response is observed and Gompertz functions could be fitted to the relative growth increments (Fig. 5; Table 2). The peak of xylem growth for all individuals

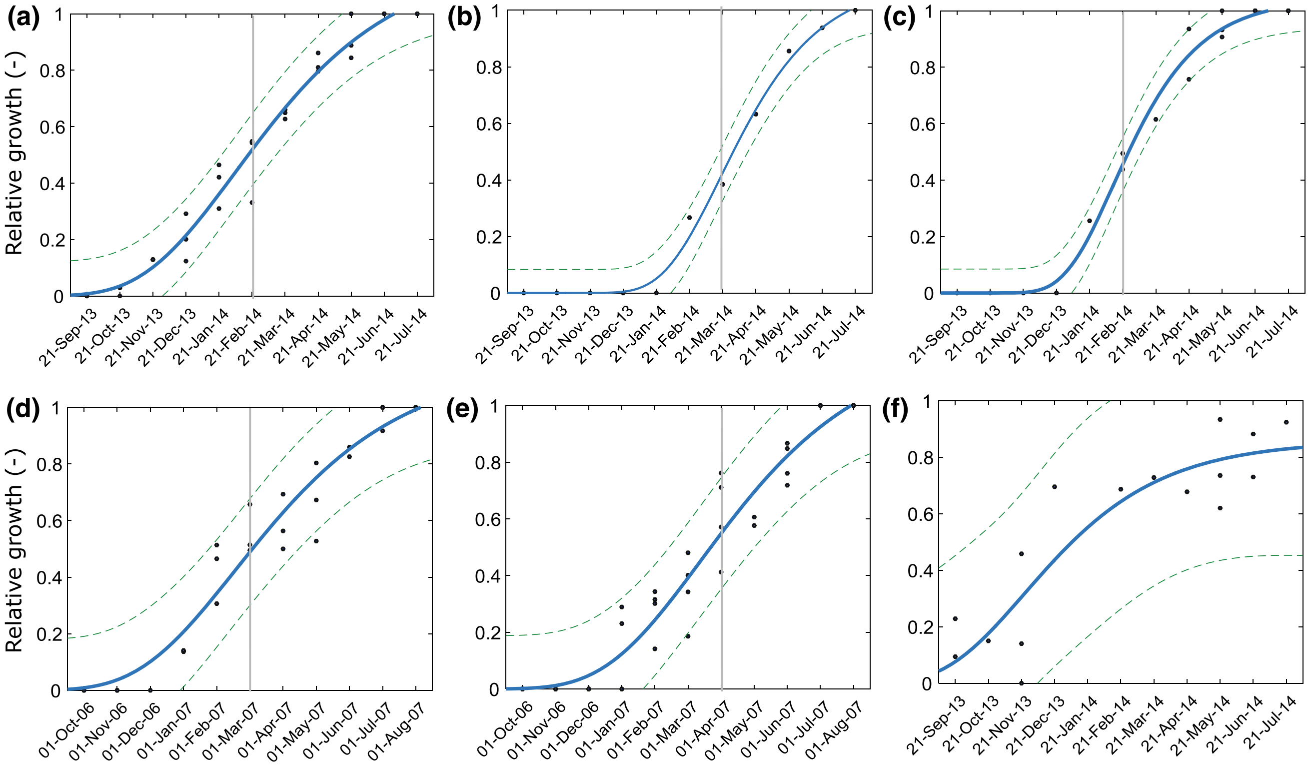


Fig. 5 Intra-annual relative growth for **a** *A. ochroleuca*, **b** *C. paniculata*, **c** *G. suaveolens,* and **f** *X. wilwerthii* for the growth season 2013–2014. For **d** *A. ochroleuca* and **e** *C. paniculata,* growth curves of the season 2006–2007 were available as well. *Solid lines* represent the Gompertz fit, *dashed lines* the 95 % prediction interval. *Vertical lines* indicate the point of inflection, i.e., the maximum growth rate

Table 2 Statistical parameters of the Gompertz fit

Species	Year	A (95 % PI)	B (95 % PI)	C (95 % PI)	SSE	*R* square	DFE	Adj *R*-sq	RMSE
A. ochroleuca	2013–2014	1.20 (1.03, 1.36)	1.98 (1.58, 2.38)	0.36 (0.26, 0.46)	0.10	0.98	27	0.97	0.06
	2006–2007	1.12 (0.93, 1.30)	2.08 (1.48, 2.67)	0.38 (0.25, 0.51)	0.20	0.96	30	0.96	0.82
G. suaveolens	2013–2014	1.04 (0.989, 1.10)	3.88 (3.09, 4.67)	0.68 (0.54, 0.82)	0.04	0.99	21	0.99	0.04
C. paniculata	2013–2014	1.11 (0.96, 1.26)	4.04 (2.88, 5.20)	0.58 (0.40 0.77)	0.01	0.99	8	0.99	0.04
	2006–2007	1.23 (0.94, 1.51)	2.23 (1.60, 2.85)	0.35 (0.21, 0.49)	0.30	0.94	35	0.94	0.09
X. wilwerthii	2013–2014	0.86 (0.59, 1.13)	1.31 (0.08, 2.54	0.43 (−0.05, 0.90)	0.32	0.79	13	0.76	0.16
	2006–2007	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)

PI prediction interval, *SSE* sum of squared errors of prediction, *R square* coefficient of determination, *DFE* degrees-of-freedom for error, *Adj R square* adjusted coefficient of determination, *RMSE* root -mean-square error seems to be more or less synchronous, around February–March. All individuals of *A. ochroleuca* showed a similar intra-annual growth pattern, starting between September and October 2013 and ending between May and June 2014 (Fig. 5a).

Three individuals of *G. suaveolens* also showed similar intra-annual wood formation, starting between December and January (Fig. 5c). Despite some wedging in the previous rings, a complete ring was formed during 2013–2014 (Fig. 4b). Yet, one *G. suaveolens* individual had clear wedging with pinning 4, 5, 6, and 7 occurring on an absent ring (Fig. 5c). Stem disks, fortunately, allowed to interpret this wedging. The remainder of the pinnings on this individual was included for the Gompertz fitting.

We could only establish a growth curve of one *C. paniculata* individual (Fig. 5b). This individual only starts growing in January and has a substantial growth lag compared with the other species in 2013–2014.

For *A. ochroleuca*, the growing range of 2006–2007 is of the same order as observed in the season 2013–2014 (Fig. 5a, d). However, growth of *C. paniculata* in 2006–2007 started growing much earlier compared with the season 2013–2014 (Fig. 5b, e), which is similar to the curve of *A. ochroleuca* (Fig. 5b vs. Fig. 5e).

Intra-individual growth: ring anomalies

In addition to wedging for the *G. suaveolens* sample, more extreme cases of ring anomalies occur: two *C. paniculata* individuals even have no discernible growth, while only wound-induced tissue is observed (Table 2; Figs. 6a, 7), resulting in a missing ring. The extent of the wound-

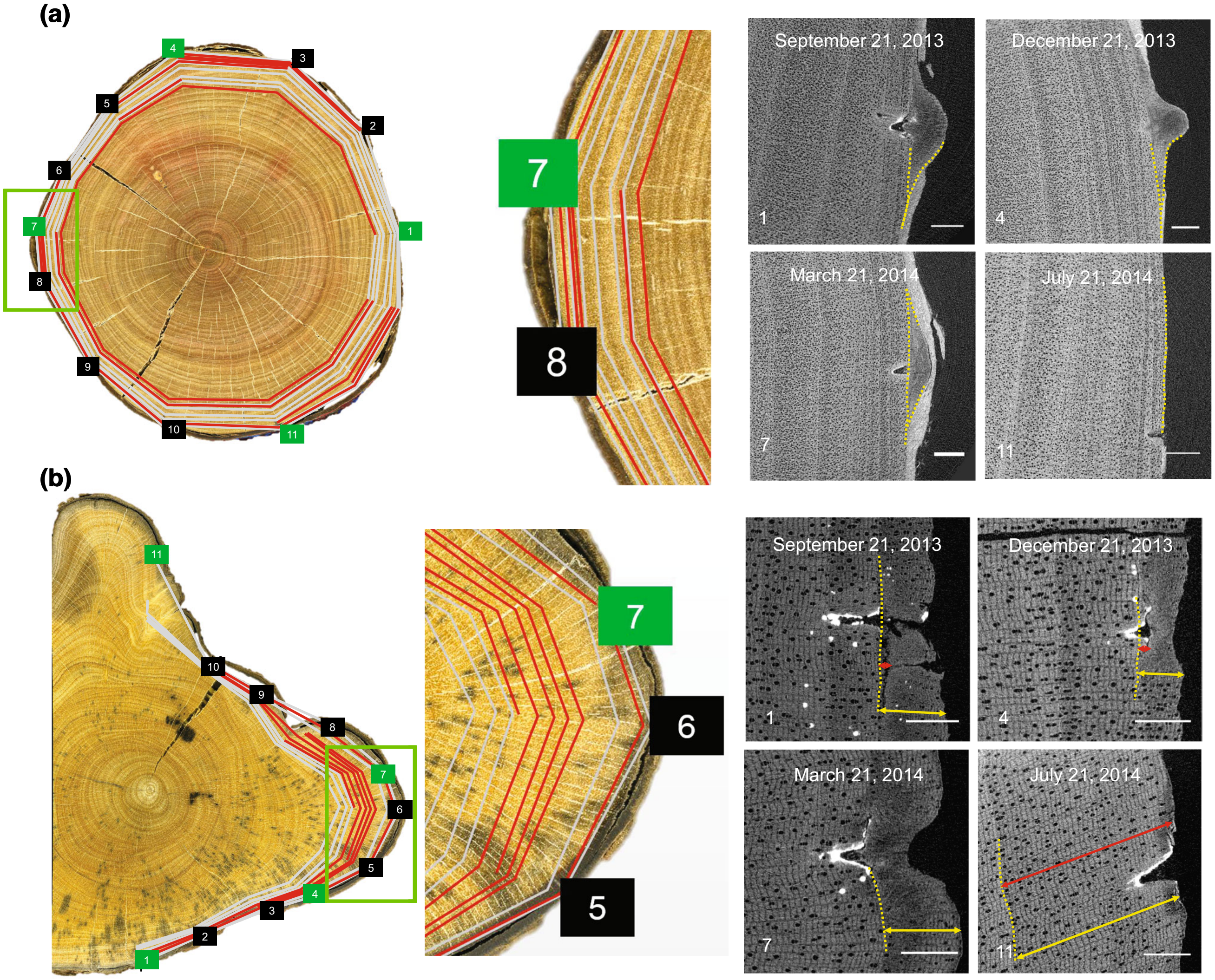


Fig. 6 Stem disk images with digitized contours of the last 5–10 rings for **a** *C. paniculata* showing only wound-induced xylem growth, and **b** *X. wilwerthii* showing circumferentially variable intra-annual growth. *White lines* represent normal rings; *red lines* indicate wedging rings. For **b,** measurements were only performed on a section of the total circumference. *Scale bar* 1 mm

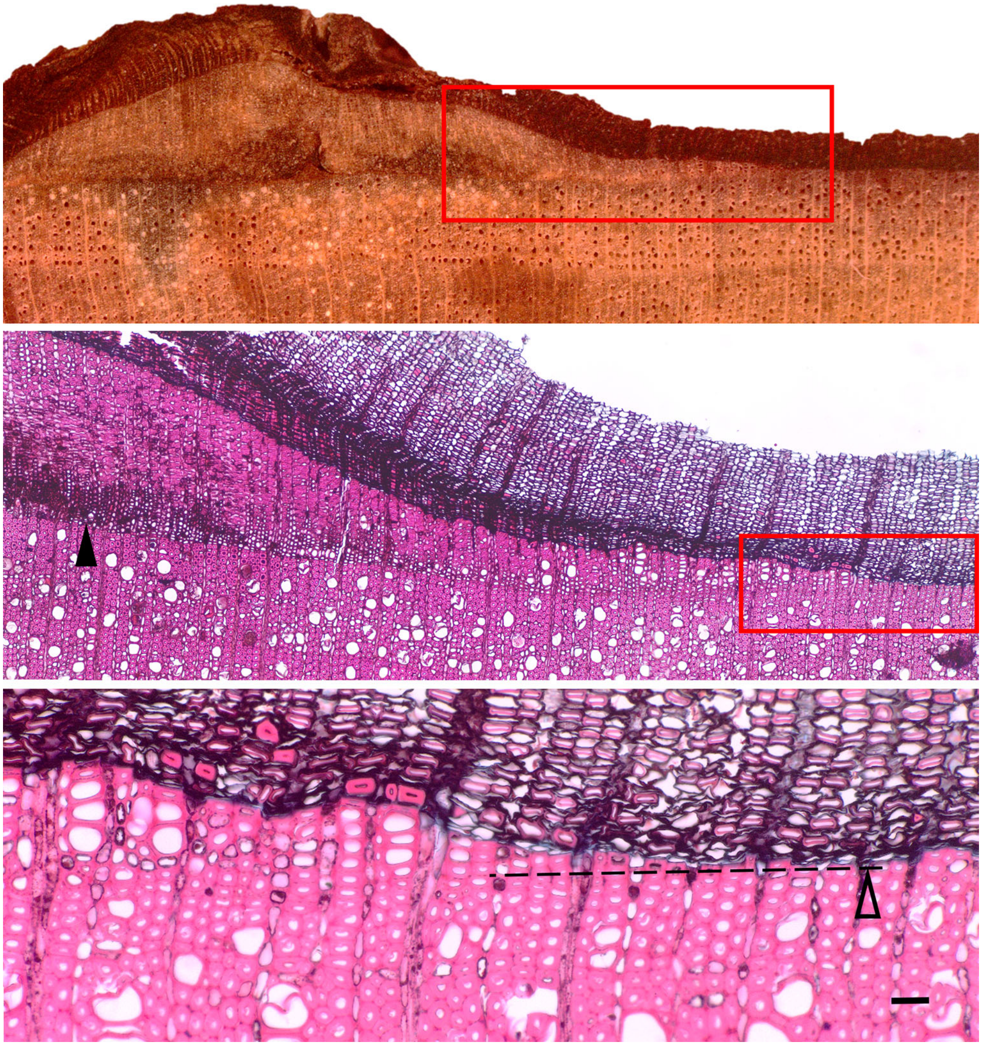


Fig. 7 *C. paniculata* sample with a pinning from September 2013. Only pinning-induced local growth occurred for the entire growth season, which means that no ring was formed. *Full arrow* indicates callus formation above the pinning wound, *empty arrow* shows onset of wound-induced xylem, *dashed line* indicates the previous growth ring boundary. *Scale bar* 100 µm

induced xylem clearly shows that the tree still has a living cambium, despite not forming any detectable xylem growth elsewhere on the stem disk.

Next to wedging, a significant circumferential variability hampers proper analysis of cambial pinnings for the *X. wilwerthii* samples from 2013–2014. This variability is seen on the digitized stem disks as highly variable ring width (Fig. 6b).

Although radial growth could be measured, the circumferential variability in intra-annual growth rate hampered the construction of a consistent intra-annual curve (Fig. 5f), even if relative growth was always present (X-ray images Fig. 6b). Fitting of a Gompertz model was possible, but model quality parameters are considerably lower (Table 2).

Discussion

Ring digitization to account for anomalies and X-ray CT for measuring intra-annual growth

The digitization of stem disks allows to assess the circumferential variability on the stem disks, similar to the annual ring diagrams described in Larson (1994). It is a convenient way to identify matching rings and anomalies along the entire circumference, and is of high importance for trees in tropical regions (Latte et al. 2015). X-ray CT allows to assess the wound tissue in 3D, before sanding or sectioning, which might erase the pinning spot and could lead to sample loss.

Species-specific and intra-species variation in intra-annual response

The early onset of the *A. ochroleuca* data concurs with earlier investigations based on monthly diameter measurements (Couralet et al. 2010). The most significant observation for 2013–2014 is the late onset of growth for *G. suaveolens* and *C. paniculata*. For *G. suaveolens,* this observation is consistent for the three individuals, whereas for *C. paniculata,* only one individual has a late onset of growth, while the other two individuals only show wound-induced cambial activity during the entire growth season. Some wedging was observed for *G. suaveolens*, but for all individuals, a growth curve could be established, as this species is known to have dendrochronological potential (Worbes et al. 2003). For *C. paniculata*, the observed late onset concurs with Couralet et al. (2010), based on the tree-ring analysis, where the species reacts on late-season climatic events.

Combined observations of phenology and wood formation are key in understanding tree-growth response in temperate regions (Michelot et al. 2012). Although no direct connection between flowering and wood formation has been found yet in the tropics, phenology monitoring of these evergreen species could tell whether leaf and cambium phenology are synchronous. A forest-wide bimodal flowering pattern based on data from Couralet et al. (2013) could explain the differing growing onsets. For *A. ochroleuca* and *G. suaveolens*, the respectively early and late onset coincide with the flowering pattern seen in Fig. 8. This bimodality concurs with the findings of Borchert et al. (2005).

However, there are large differences in onsets between the season 2013–2014 and 2006–2007 for *C. paniculata.* Naturally, these trees were sampled from a different site and a different season. Thus, other factors, such as site, as well as social status of the individual due to competition can also have a considerable influence on the onset (Rathgeber et al. 2011). Increasing competition can lead to later onsets and smaller ring width. Ultimately, a further lack of assimilates due to this competition will hamper proper ring development along the entire circumference, which leads to intra-individual variations.

Intra-individual variation: wedging, zero xylem growth at the stem base, and circumferential variation

The studied understory species show different ring anomalies, ranging from wedging to the extreme case of zero growth, resulting in missing rings and concurring with the zero radial increment of certain individuals observed by Couralet et al. (2010) at the sampled height.

Unfortunately, a little information exists on the causes of these ring anomalies and their drivers in the tropics. The only literature at hand comes from temperate and boreal regions, with trees growing under climatic stress. The understory position of the trees in this study, however, also gives rise to stresses, such as changing light conditions (Worbes and Fichtler 2010) and a small crown due to competition, thus provoking limited availability of assimilates which could be the cause of an increased wedging. Indeed, Wilmking et al. (2012) mention that tree species which are limited in resources start forming wedging rings, and, consecutively, higher stress levels cause zero xylem growth at the stem base resulting in missing rings. Using digitized stem disks, we, indeed, noticed that multiple wedging rings occur in growth years prior to the pinning season, which could indicate an increase in stress eventually provoking zero growth of the *C. paniculata* samples from 2013–2014. Finally, it is known that missing rings are more frequent in older and larger trees (Novak et al. 2016), but no direct link could be found here between tree size and frequency of wedging for the samples investigated

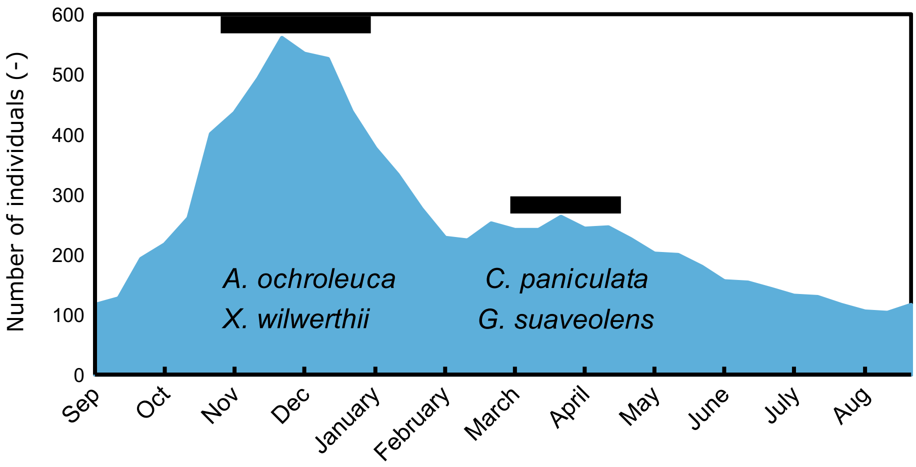


Fig. 8 Forest-wide bimodal flowering pattern of the Luki reserve (data adopted from Couralet et al. 2013)

(Table 1). It should be mentioned, however, that tree-ring studies along the stem axis (Fritts 1976; Leland et al. 2016), as well as vertically consecutive tree diagrams (Larson 1994) show that tree rings can still form further upwards in the canopy, even though missing rings are present at the sampled height.

Our observation of wound-induced xylem formation adjacent to the needle insertion (Fig. 7), despite the zero growth elsewhere along the circumference, gives indirect evidence of ring formation further upwards in the stem axis. The phloem fails to provide assimilates for actual xylem formation at the stem basis, but is still able to form wound-induced xylem. This could imply that the cambium is inactive but can be activated by wounding, resulting in local growth (Copini et al. 2014), a term which we will refer to instead of wound-induced xylem growth. In the latter study, local growth was measurable at the end of the growth season while being masked earlier in the season. However, in our study, the zero growth samples show local growth all year round. What is even more, in some cases, wood formation does not occur around the wound-induced zone, yet the ring tends to reappear further away from the pinning along the circumference. Hypothetically speaking, when resources are limited, a local triggering of a hormonal response (Copini et al. 2014) could lead to a compensational mechanism with a local decrease in xylem growth in the area adjacent to the local growth zone.

When excessive circumferential variability in growth rate exists, however, such as for *X. wilwerthii* (Figs. 5f, 6b), accurate intra-annual xylogenesis curves cannot be obtained. Abundant wedging occurs in the previous rings, but not during the pinning experiment. Despite corrections applied here, as reported in Seo et al. (2007), the intra-annual growth seems to be too irregular, making it difficult to see in what part of the season *X. wilwerthii* is growing, emphasizing the remarkable asymmetrical plasticity of the cambium around the circumference. This is a major challenge in the tree-ring analysis (Cherubini et al. 2013), as eccentric stems might exhibit circumferentially differing tree response (Buras and Wilmking 2014).

Conclusion

This is the first study of intra-annual xylem growth on understory species in tropical Africa. Stem disks were digitized to map and track ring anomalies, such as wedging. X-ray Computed Tomography proves to be an efficient tool for measuring relative growth, and, if needed, provides a guided selection for micro-sectioning or sanding. We show varying intra-annual growth patterns, which imply species-specific responses on climate. The bimodal flowering pattern might be an explanation for the intra-annual growth differences between species. However, these responses can also be influenced by other factors, such as site and competition. As seasonality is weaker in the tropics compared with more temperate regions, the impact of aforementioned factors can be considerable. The striking observation that so many sampled pinnings showed no xylem growth at the stem base, apart from wound-induced local growth, emphasizes the complexity of tropical wood formation and resource availability of these species. There is a need for additional thorough research on the phenomena, such as local growth and wound-induced wood formation. The aspect of wedging and missing rings is well documented in temperate and arctic regions. However, it is rarely studied in the tropics, though it is being widely acknowledged as a problem in the tropical tree-ring analysis. Therefore, pinning and related studies are essential to explain difficulties in the tree-ring analyses, especially in the African forests, where many species remain understudied.

Author contribution statement TDM, BAI, JVA, HB, and JVdB, have designed and optimized the study. TDM set up the experiment; TDM, BAI, and SM sampled the wood materials. Measurements were performed by SM and TDM supervised by JVdB, HB, and JVA. JD provided comments on the X-ray CT setup, and analysis and manuscript by TDM, with the support of all co-authors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Amobi CC (1973) Periodicity of wood formation in some trees of lowland rainforest in Nigeria. Ann Bot 37:211–218

Ballesteros-Cánovas JA, Stoffel M, Guardiola-Albert C (2015) XRCT images and variograms reveal 3D changes in wood density of riparian trees affected by floods. Trees 29:1115–1126

Battipaglia G, De Micco V, Brand WA, Linke P, Aronne G, Saurer M, Cherubini P (2010) Variations of vessel diameter and δ13C in false rings of *Arbutus unedo* L. reflect different environmental conditions. New Phytol 188:1099–1112

Bauch J, Quiros L, Noldt G, Schmidt P (2006) Study on the wood anatomy, annual wood increment and intra-annual growth dynamics of *Podocarpus oleifolius* var. *macrostachyus* from Costa Rica. J Appl Bot Food Qual 80:19–24

Borchert R, Renner SS, Calle Z, Navarrete D, Tye A, Gautier L, Spichiger R, von Hildebrand P (2005) Photoperiodic induction of synchronous flowering near the Equator. Nature 433:627–629

Buras A, Wilmking M (2014) Straight lines or eccentric eggs? A comparison of radial and spatial ring width measurements and its implications for climate transfer functions. Dendrochronologia 32:313–326

Camarero JJ, Olano JM, Parras A (2010) Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. The New phytologist 185:471–480

Cherubini P, Humbel T, Beeckman H, Gärtner H, Mannes D, Pearson C, Schoch W, Tognetti R, Lev-Yadun S (2013) Olive tree-ring problematic dating: a comparative analysis on santorini (Greece). PLoS One 8:1–5

Chowdhury MQ, Kitin P, de Ridder M, Delvaux C, Beeckman H (2015) Cambial dormancy induced growth rings in *Heritiera fomes* Buch.- Ham.: a proxy for exploring the dynamics of Sundarbans, Bangladesh. Trees 30:227–239

Copini P, Den Ouden J, Decuyper M, Mohren GMJ, Loomans AJM (2014) Early wound reactions of Japanese maple during winter dormancy : the effect of two contrasting temperature regimes. AoB PLANTS 6:plu059. doi:10.1093/aobpla/plu059

Couralet C (2010) Community dynamics, phenology and growth of tropical trees in the rain forest Reserve of Luki, Democratic Republic of Congo. Ph.D. thesis. Faculty of Bioscience Engineering, Ghent University, Belgium

Couralet C, Sterck FJ, Sass-Klaassen U, Van Acker J, Beeckman H (2010) Species-specific growth responses to climate variations in understory trees of a central african rain forest. Biotropica 42:503–511

Couralet C, Van den Bulcke J, Ngoma LM, Van Acker J, Beeckman H (2013) Phenology in functional groups of central african rainforest trees. J Trop Forest Sci 25:361–374

Cuny HE, Rathgeber CBK, Kiessé TS, Hartmann FP, Barbeito I, Fournier M (2013) Generalized additive models reveal the intrinsic complexity of wood formation dynamics. J Exp Bot 64:1983–1994

Cuny HE, Rathgeber CBK, Frank D, Fonti P, Mäkinen H, Prislan P, Rossi S, del Castillo EM, Campelo F, Vavrčík H, Camarero JJ, Bryukhanova MV, Jyske T, Gričar J, Gryc V, De Luis M, Vieira J, Čufar K, Kirdyanov AV, Oberhuber W, Treml V, Huang J-G, Li X, Swidrak I, Deslauriers A, Liang E, Nöjd P, Gruber A, Nabais C, Morin H, Krause C, King G, Fournier M (2015) Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nature Plants 1:15160

Deslauriers A, Morin H, Urbinati C, Carrer M (2003) Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Québec (Canada). Trees 17:477–484. doi:10.1007/s00468-003-0260-4

Dié A, Kitin P, Kouamé FN, Van den Bulcke J, Van Acker J, Beeckman H (2012) Fluctuations of cambial activity in relation to precipitation result in annual rings and intra-annual growth zones of xylem and phloem in teak (*Tectona grandis*) in Ivory Coast. Ann Bot 110:861–873

Dierick M, Van Loo D, Masschaele B, Van den Bulcke J, Van Acker J, Cnudde V, Van Hoorebeke L (2014) Recent micro-CT scanner developments at UGCT. Nucl Instrum Methods Phys Res Sect B 324:35–40

Dünisch O, Bauch J, Gasparotto L (2002) Formation of increment zones and intra-annual growth dynamics in the xylem of *Swietenia macrophylla*, *Carapa guianensis*, and *Cedrela odorata* (Meliaceae). IAWA Journal 23:101–119

Fouarge J, Gérard G (1964) Bois du Mayumbe. Institut National pour l'Etude Agronomique du Congo belge (INEAC), Brussels

Fritts HC (1976) Tree rings and climate. Academic Press, New York

Gebrekirstos A, Bräuning A, Sass-klassen U, Mbow C (2014) Opportunities and applications of dendrochronology in Africa. Curr Opin Environ Sustain 6:48–53

Gompertz B (1825) On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Philos Trans R Soc Lond 115:513–583

Krepkowski J, Bräuning A, Gebrekirstos A (2012) Growth dynamics and potential for cross-dating and multi-century climate reconstruction of *Podocarpus falcatus* in Ethiopia. Dendrochronologia 30:257–265

Larson PR (1994) The vascular cambium: development and structure. Springer, Berlin

Latte N, Beeckman H, Bauwens S, Bonnet S, Lejeune P (2015) A novel procedure to measure shrinkage-free tree-rings from very large wood samples combining photogrammetry, high-resolution image processing, and GIS tools. Dendrochronologia 34:24–28

Leland C, Hom J, Skowronski N, Ledig FT, Krusic J, Cook ER, Martin-benito D, Martin-fernandez J (2016) Missing rings, synchronous growth, and ecological disturbance in a 36-year pitch pine (*Pinus rigida*) provenance study. PLoS One 11:e0154730

Lisi CS, Tomazello FM, Botosso PC, Roig FA, Maria VRB, Ferreira-Fedele L, Voigt ARA (2008) Tree-ring formation, radial increment periodicity, and phenology of tree species from a seasonal semi-deciduous forest in southeast Brazil. IAWA J 29:189–207

Lubini CA (1997) La végétation de la Réserve de Biosphère de Luki au Mayombe (Zaïre). Jardin Botanique National de Belgique, Meise

Lupi C, Rossi S, Vieira J, Morin H, Deslauriers A (2013) Assessment of xylem phenology: a first attempt to verify its accuracy and precision. Tree Physiol 34:87–93

Mäkinen H, Seo JW, Nöjd P, Schmitt U, Jalkanen R (2008) Seasonal dynamics of wood formation: a comparison between pinning, microcoring and dendrometer measurements. Eur J Forest Res 127:235–245

Marcati CR (2006) Seasonal variation in wood formation of *Cedrela fissilis* (Meliaceae). IAWA J 27:199–211

Mariaux A (1967) Les cernes dans les bois tropicaux africains, nature et périodicité: peuvent-ils révéler l'âge des arbres? Bois et Forêts des Tropiques 113:3–14

Michelot A, Simard S, Rathgeber C, Dufrêne E, Damesin C (2012) Comparing the intra-annual wood formation of three European species (*Fagus sylvatica, Quercus petraea* and *Pinus sylvestris*) as related to leaf phenology and non-structural carbohydrate dynamics. Tree Physiol 32:1033–1045

Morel H, Mangenet T, Beauchêne J, Ruelle J, Nicolini E, Heuret P, Thibaut B (2015) Seasonal variations in phenological traits: leaf shedding and cambial activity in *Parkia nitida* Miq. and *Parkia velutina* Benoist (Fabaceae) in tropical rainforest. Trees 29:973–984

Novak K, de Luis M, Saz MA, Longares LA, Notivoli S, Raventós J, Čufar K, Gričar J, Di Filippo A, Piovesan G, Rathgeber CB, Papadopoulos A, Smith KT (2016) Missing rings in *Pinus halepensis*—the missing link to relate the tree-ring record to extreme climatic events. Front Plant Sci. doi:10.3389/fpls.2016.00727

Olson DM et al (2001) Terrestrial ecoregions of the world: a new map of life on Earth. Bioscience 51:933–938

Peel MC, Finlayson BL, McMahon TA (2007) Updated world map of the Köppen-Geiger climate classification. Hydrol Earth Syst Sci 11:1633–1644

Pendje G, Baya M (1992) La réserve de biosphère de Luki (Mayombe, Zaïre). Patrimoine floristique et faunistique en péril. PNUD-UNESCO-MAB, Paris

Rathgeber CBK, Rossi S, Bontemps JD (2011) Cambial activity related to tree size in a mature silver-fir plantation. Ann Bot 108:429–438

Remane IAD, Therrell MD (2015) Dendrochronological potential of Millettia stuhlmannii in Mozambique. Trees 29:729–736

Rossi S, Deslauriers A, Morin H (2003) Application of the Gompertz equation for the study of xylem cell development. Dendrochronologia 21:33–39

Rossi S, Deslauriers A, Anfodillo T (2006) Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the Alpine timberline. IAWA Journal 27:383–394

Rossi S, Anfodillo T, Cufar K, Cuny HE, Deslauriers A, Fonti P, Frank D, Gricar J, Gruber A, King GM, Krause C, Morin H, Oberhuber W, Prislan P, Rathgeber CBK (2013) A meta-analysis of cambium phenology and growth: linear and non-linear patterns in conifers of the Northern Hemisphere. Ann Bot 112:1911–1920

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ : 25 years of image analysis. Nat Methods 9:671–675

Senechal J, Kabala M, Fournier F (1989) Revue des connaissances sur le Mayombe. UNESCO, Paris

Seo J-W, Eckstein D, Schmitt U (2007) The pinning method: from pinning to data preparation. Dendrochronologia 25:79–86

Stoffel M, Klinkmüller M (2013) 3D analysis of anatomical reactions in conifers after mechanical wounding: first qualitative insights from X-ray computed tomography. Trees 27:1805–1811

Trouet V, Mukelabai M, Verheyden A, Beeckman H (2012) Cambial growth season of brevi-deciduous Brachystegia spiciformis trees from south central Africa restricted to less than four months. PLoS One 7:e47364

van der Werf GW, Sass-Klaassen UGW, Mohren GMJ (2007) The impact of the 2003 summer drought on the intra-annual growth pattern of beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) on a dry site in the Netherlands. Dendrochronologia 25:103–112

Verheyden A, Kairo Gitundu J, Beeckman H, Koedam N (2004) Growth rings, growth ring formation and age determination in the mangrove *Rhizophora mucronata*. Ann Bot 94:59–66

Vlassenbroeck J, Dierick M, Masschaele B, Cnudde V, Van Hoorebeke L, Jacobs P (2007) Software tools for quantification of X-ray microtomography at the UGCT. Nucl Instrum Methods Phys Res, Sect A 580:442–445

White F (1983) The vegetation of Africa. Natural Resources Research, vol 20. UNESCO, Paris

Wilmking M, Hallinger M, Van Bogaert R, Kyncl T, Babst F, Hahne W, Juday GP, De Luis M, Novak K, Völlm C (2012) Continuously missing outer rings in woody plants at their distributional margins. Dendrochronologia 30:213–222

Winsor CP (1932) The Gompertz curve as a growth curve. Proc Natl Acad Sci USA 18:1–8

Wolter KE (1968) Notes: a new method for marking xylem growth. Forest Sci 14:102–104

Worbes M (2002) One hundred years of tree-ring research in the tropics—a brief history and an outlook to future challenges. Dendrochronologia 20:217–231

Worbes M, Fichtler E (2010) Wood anatomy and tree-ring structure and their importance for tropical dendrochronology. In: Junk WJ, Piedade MTF, Wittmann F, Schöngart J, Parolin P (eds) Amazonian floodplain forests: ecophysiology, biodiversity and sustainable management. Springer, Heidelberg, pp 329–346

Worbes M, Staschel R, Roloff A, Junk W (2003) Tree ring analysis reveals age structure, dynamics and wood production of a natural forest stand in Cameroon. For Ecol Manage 173:105–123

Zeide B (1993) Analysis of growth equations. Forest Sci 39:594–616