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Cambial activity in dry and rainy season on branches from woody species growing in Brazilian Cerrado

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ABSTRACT

Seasonal cambial activity was investigated in one- to three-year-old branch modules (branch constructional units) of ten woody species from cerrado *sensu stricto*, a savanna-like ecosystem, of southern Brazil. Relationships between cambial activity and environmental factors (precipitation, temperature, day length) and leaf production were tested using generalized linear mixed model. Regardless the plant habit and leaf shedding patterns, cambial activity (about 5 months) corresponded to the core of the rainy season (when at least 66% of the annual precipitation had been reached), even though there was some delay or advance in the timing of cambial activity depending on the branch age or species. Cambial activity began in the dry season and ceased before the end of rainy season. Although cambial activity has been positively correlated with day length, precipitation, temperature, and budding for all species, day length, among the assessed factors, seems to be the best predictor of onset and cessation of cambial activity in the branch modules of cerrado species.

1. Introduction

Studies on seasonal activity of the vascular cambium and how that activity is influenced by environmental factors (temperature, rainfall, soil moisture, day length) and its relation to plant attributes (phenophases and habit) provide critical data for understanding the growth dynamics of trees (Jacoby, 1989; Kozlowski et al., 1991).

It is generally assumed that cambial activity in temperate trees is influenced primarily by temperature and day length, whereas in tropical trees rainfall and available soil water are more important factors (Evert and Eichhorn, 2006). A distinct seasonality in cambial activity has been reported in many tropical and subtropical regions from South America that experience severe annual dry seasons (Callado et al., 2013). For tropical trees, the induction of cambial dormancy has been attributed to an annual dry season lasting from 2 to 3 months with a rainfall of less than 60 mm (Worbes, 1995). Worbes' observation seems to be true for some species, as cambial activity has been related to precipitation for a number of tropical species (see Dave and Rao, 1982b; Rajput and Rao, 2001; Tomazello-Filho and Cardoso, 1999; Rao and Rajput, 1999, 2000, 2001; Aref et al., 2014). In fact, in some semi-deciduous seasonal forest species as *Cedrela fissilis* (Marcati et al., 2006) and *Schizolobium parahyba* (Marcati et al., 2008) the active period of the cambium coincided with the rainy season, and the dormant period with the dry season.

Other studies on the initiation and cessation of cambial activity in tropical and subtropical regions that experience severe annual dry seasons demonstrate the relevance of factors other than only rainfall. Kokutse et al. (2010) reported that in the main stem of teak (*Tectona grandis*, a deciduous tree) cultivated in central Togo, West Africa, there was a correlation between initiation of cambial activity and rainfall at the beginning of the rainy season but annual ring width depended strongly on mean temperature during the rainy season. In a subtropical rainforest of Mexico, leaf initiation and cambial activity were found to be associated with maximum temperature and day length in branches (Yáñez-Espinosa et al., 2006, 2010). Trouet et al. (2012) studying cambial growth periodicity in the main stem of brevi-deciduous *Brachystegia spiciformis* trees from seasonally dry miombo woodland of southern Africa

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observed that the onset and end of cambial growth was not concurrent with the onset and end of the rainy season. They found a relatively short (three to four months) cambial growth season that corresponded to the core of the rainy season. According to Borchert (1999), although the inhibition of plant growth by drought stress is well established in tree species growing in tropical climates with a long, severe dry season, responses to seasonal drought vary widely among such trees, and their annual development is not well synchronized by climatic seasonality. According to the author, in deciduous trees growing at microsites with low soil moisture storage, phenology and cambial growth are well correlated with each other and with seasonal rainfall, and most trees have distinct annual rings. Phenology and cambial growth are progressively uncoupled from climatic seasonality in brevi-deciduous and evergreen trees growing at microsites with large soil water reserves that buffer trees against seasonal drought and thus may prevent the formation of distinct annual rings (Borchert, 1999). As per Kozlowski (1971), caution should be exercised in ascribing the cause and effect relations to correlation analysis of cambial growth and individual factors. It is important to emphasize that most of these studies were performed in the main stem of trees, except the studies by Yáñez-Espinosa et al. (2006, 2010), which was carried out on branches.

The Brazilian Cerrado, a savanna-like ecosystem, is characterized by a strongly seasonal climate with distinctive wet and dry seasons; soils are deep and well drained, acidic, extremely low in available nutrients and with high aluminum content (Oliveira and Marquis, 2002). This tropical savanna is composed by several vegetation types, from open grasslands to dense woodlands (Oliveira-Filho and Ratter, 2002), and the function and structure of this ecosystem are influenced by fire frequency, soil fertility, water availability and herbivory (Gottsberger and Silberbauer-Gottsberger, 2006). The cerrado *sensu stricto* is dominated by tortuous treelets often 3–8 m tall and shrubs giving more than 30% crown cover but with still a fair amount of herbaceous vegetation between them (Oliveira-Filho and Ratter, 2002). As Cerrado species experience a seasonal climate we presume that this condition favors woody species to have a seasonal cambial activity; however, studies on cambial activity for these species are lacking.

In this work we studied ten representative woody species from cerrado *sensu stricto* aiming at relating cambial activity with environmental factors and plant attributes in order to look for growth patterns for this ecosystem. We also provide a comparison among different branch ages in order to evaluate the age influence in the cambial dynamics.

2. Materials and methods

This study was carried out on ten species (*Annona coriacea* Mart., Annonaceae; *Gochnatia barrosoae* Cabrera and *Piptocarpha rotundifolia* (Less.) Baker, Asteraceae; *Diospyros hispida* A.DC., Ebenaceae; *Bowdichia virgilioides* Kunth, *Leptolobium elegans* Vogel and *Machaerium villosum* Vogel, Leguminosae-Faboideae; *Byrsonima verbascifolia* (L.) DC., Malpighiaceae; *Roupala montana* Aubl., Proteaceae; and *Pouteria torta* (Mart.) Radlk., Sapotaceae) with different habits (tree and shrub), and leaf shedding pattern (evergreen and deciduous) (Table 1) growing in a remnant of cerrado *sensu stricto* located in Pratânia municipality (23°02′55.5″S and 48°31′26.1″W), São Paulo State, Brazil, at an altitude of approximately 700 m.

To test if cambial activity in the ten species is related to rainfall, we collected branches at the beginning of the rainy season (October 16, 2004), during the rainy season (March 18, 2004 and February 02, 2005), during the dry season (June 22, 2004 and Jun 03, 2005), and at the end of the dry season (September 15, 2005) (Fig. 1). We have performed the sampling in intervals of 3–4 months during each of the two years. Three or four individuals were sampled for each species at each collection date, depending on how many individuals were found in the studied area. Each individual was considered a sampling unit. We collected one branch from the same individuals every sampling period. Then, each branch was partitionated to be analyzed separately in one-, two-, and three-year-old modules (modules are considered as branch constructional units, *sensu* Bell, 1991). Terminal bud scale scars were used to determine the age of each branch module (Raven et al., 1999).

Each branch module was separately fixed in CRAF III (10% chromic acid, glacial acetic acid, 37% formaldehyde and distilled water; Berlyn and Miksche, 1976) for about 10 days.

The oldest portion of each module (1-, 2-, 3-year-old) was cut in a sliding microtome (15 μm thickness) in order to detect cambial activity in the whole circumference. The sections, about 15 μm thick, were clarified with sodium hypochlorite (50%) then double stained with aqueous 1% safranin (Bukatsch, 1972) and aqueous 1% astra blue (Roeser, 1972) (1:9). Sectioned material was mounted in synthetic resin (Entellan®).

Then, small specimens (ca. 0.5 cm^3) from the oldest portion of each module were dehydrated and embedded in plastic resin (Historesin®) (Bennett, 1976) in order to study the cambial activity in detail. Transverse and longitudinal sections about 5 μm thick were cut with a rotary microtome, using a steel knife. The sections were stained with 1% toluidine blue in acetate buffer, pH 4.7, producing a metachromatic stain (O'Brien et al., 1964).

We used the terminology proposed by Esau (1967) for cambial zone. We identified, in transverse sections, the radial rows of initial cells plus derivatives between differentiating/differentiated xylem and phloem cells. Cambial activity was identified by the presence of very thin, recently formed periclinal cell walls.

We divided cambial status into three categories: (i) dormant (D), when there was no recently formed periclinal cell walls or mitotic figures (Fig. 2) in the whole circumference of the branches; (ii) beginning (B) or ending (E) activity (according to the chronological sequence), when we could observe very thin, recently formed periclinal cell walls (Fig. 3) in different portions of the circumference of the branches; (iii) active (A), when we could observe very thin, recently formed periclinal cell walls and mitotic figures, and a considerable amount of secondary xylem or phloem recently produced under differentiation (Fig. 4) in the whole circumference of the branches. In both categories (i) and (ii) none or very few immature secondary xylem or phloem cells could be seen adjacent to the cambial zone.

Leaf phenology was registered monthly in 2004 and 2005 by naked eye or by use of binoculars. The phenological phases were identified according to Morellato et al. (1989), but modified: (a) budding, the appearance of the red or light-green coloured leaf primordia; (b) new leaves, characterized by expanding leaf blades; (c) mature leaves, fully expanded; (d) leaf fall, when at least one of the following characteristics were observed: the leaf drops easily from the branch, presence of leaf scars at the apex of the branch, presence of voids in the canopy or leafless branches. We applied an index of intensity to the phenological phases – the intensity of phenological phases was estimated according to an interval scale varying from 0 to 3 with a 33% interval between classes (0 = absence of the characteristic, 1 = presence of the characteristic with a range from 1 to 33%, 2 = presence of the characteristic with a range from 34 to 66%, 3 = presence of the characteristic with a range from 67 to 100%).

Climate data (Fig. 1) were obtained from the Meteorological Station at the Faculdade de Ciências Agronômicas, Universidade Estadual Paulista (UNESP), Botucatu. The Meteorological Station was about 25 km far from the study site. We took mean data for the 30 days prior to the day of collection for temperature and day

Table 1
Climate data from the studied region, habit, functional group, mean height of the individuals, leaf phenology (♣ = budding; ⅄ = new leaves; ♠ = mature leaves; ▼ = leaf fall), and cambium status (B = beginning; A = active; E = ending; D = dormancy), in 1, 2, 3 year-old branches.

		2004			2005		
	Months	Mar	Jun	Oct	Feb	Jun	Sep
Precipitation	(mm)	122	35	109	66	51	35
Mean Temp	(°C)	21	16	19	22	18	18
Min Temp	(°C)	18	13	16	18	15	14
Max Temp	(°C)	27	22	25	29	24	24
Daylength	(h)	12	11	13	13	11	12
Annona coriacea		♣♠	⅄ ♠	♣⅄ ♠	♣♠▼	⅄ ♠▼	♣⅄ ♠▼
tree	1	D	D	A	E	D	B
evergreen	2	D	D	A	E	D	D
~3 m tall	3	D	D	B	E	D	D
Gochnatia barrosoae		♣⅄ ♠	⅄ ♠▼	♣⅄	♣⅄ ♠	⅄ ♠▼	♠▼
shrub	1	D	D	B	E	D	D
evergreen	2	D	D	D	D	D	D
~2.5 m tall	3	D	D	D	D	D	D
Piptocarpha rotundifolia		♠	♠▼	♣⅄	♣⅄ ♠	♠	♣⅄ ♠▼
shrub	1	D	D	A	E	D	B
evergreen	2	D	D	A	D	D	B
~2 m tall	3	D	D	A	E	D	B
Diospyros hispida		♠	♠▼	♣⅄	♠	♠▼	♣⅄ ▼
shrub	1	D	D	B	E	D	B
evergreen	2	D	D	B	E	D	B
~3 m tall	3	D	D	D	E	D	B
Bowdichia virgilioides		♣⅄ ♠	♣⅄ ♠▼	♣⅄	♣⅄ ♠	♣⅄ ♠▼	♣⅄ ♠▼
tree	1	D	D	B	E	D	B
evergreen	2	D	D	B	E	D	D
~7 m tall	3	D	D	D	D	D	D
Leptolobium elegans		♠	♠	♣⅄	♠	♠▼	▼
tree	1	D	D	A	E	D	D
deciduous	2	D	D	A	A	D	D
~3 m tall	3	D	D	A	A	D	D
Machaerium villosum		⅄ ♠	♠▼	♣⅄	♣⅄ ♠	♠	♣⅄ ♠▼
tree	1	D	D	A	D	D	B
evergreen	2	D	D	A	D	D	B
~12 m tall	3	E	D	A	E	D	B
Byrsonima verbascifolia		♣⅄ ♠	♠	⅄	♣⅄ ♠▼	⅄ ♠▼	♠▼
tree	1	D	D	A	E	D	B
evergreen	2	D	D	B	E	D	D
~5 m tall	3	D	D	B	A	D	D
Roupala montana		♠	♠▼	♣⅄	♣⅄ ♠	♠	♣⅄ ♠▼
tree	1	D	D	A	E	D	B
evergreen	2	E	D	A	A	D	B
~2 m tall	3	E	D	A	A	D	B
Pouteria torta		♠	♠▼	♣⅄	♣⅄ ♠	♠	♣⅄ ♠▼
tree	1	D	D	A	D	D	D
deciduous	2	D	D	B	D	D	D
~4 m tall	3	E	D	D	E	D	D

length, and total sum of precipitation for the 30 days prior to the day of collection.

We analyzed 1-, 2-, 3-year-old branches separately, as well as combining age classes to all species, evergreen, and deciduous species. To test the effects of environmental factors and leaf phenology on cambial activity we used a binomial generalized linear mixed model (GLMM) that accommodates the hierarchical structure of our sampling design (individuals nested within species). In the analyses ignoring age classes, the cambium was considered active if at least one of the branches was presenting cambial activity. Due to the multicollinearity among the predictor factors, we used three models to correlate cambial activity to day length, precipitation, temperature, and leaf phenology. From the min, max and mean temperatures, we chose maximum temperature based on the correlation found between cambial activity and maximum temperature obtained by Yáñez-Espinosa et al. (2006) studies. From the leaf phenology, we chose budding based on the correlation found between cambial activity and budding obtained in several studies (e.g. Dave and Rao, 1982a,b; Venugopal and Krishnamurthy, 1987; Aref et al., 2014). The most parsimonious models were determined by sequential backward removal of non-significant terms based on conditional *z* tests. Residuals were visually inspected to check for model assumptions. Models were fitted using the lme4 package (Bates et al., 2015) in the R environment (Core Team, 2014).

3. Results

We found that for the ten study species from cerrado *sensu stricto*, the sampling frequency allowed us to determine the cambial dormancy (Figs. 5–10) and activity (Figs. 11 –15) period. In general, cambial activity occurred from October 2004 to February 2005 in most branch modules of all species (Table 1, Figs. 11–15). The cambium was active for around 5 months, corresponding to the core of the rainy season (Fig. 1) even though there was some

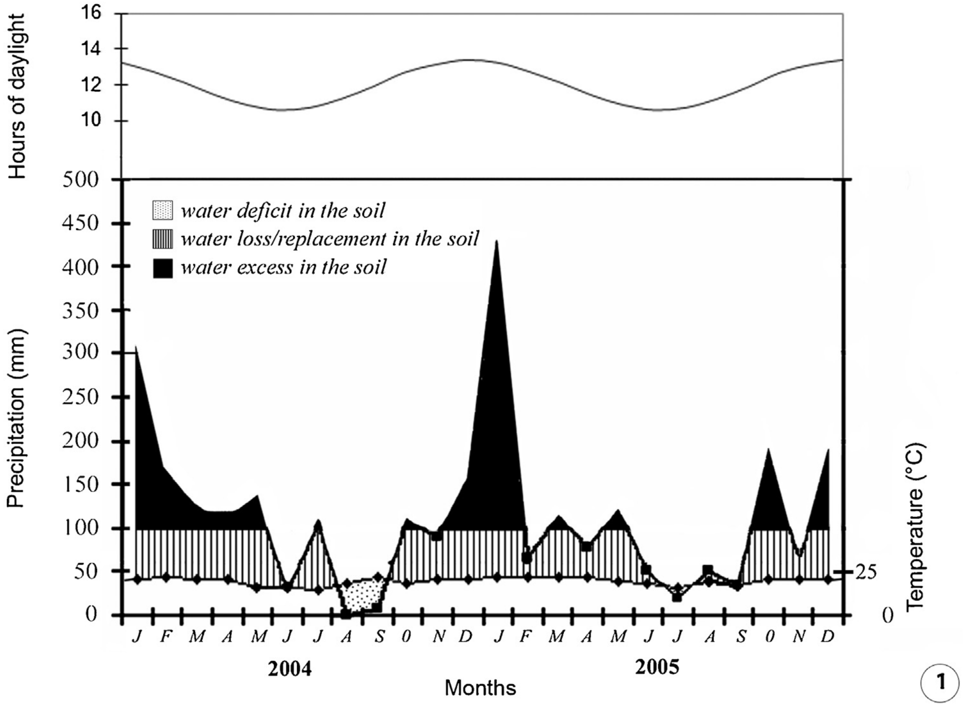
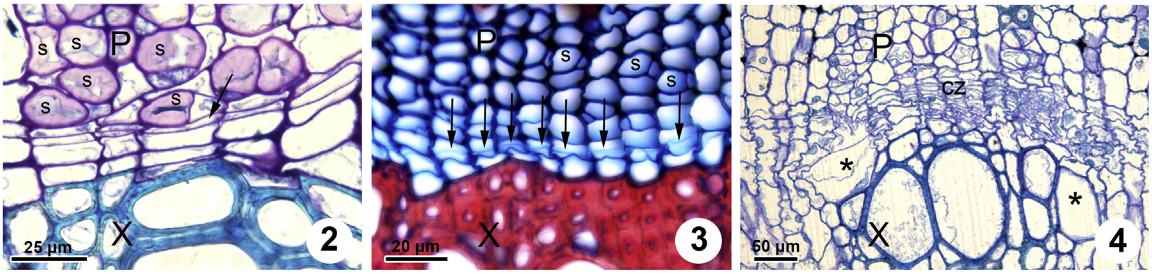


Fig. 1. Upper panel: day length at the sampling site (23° S). Lower panel: Walter climate diagram displaying the length of dry and wet season of the Cerrado at Botucatu municipality, São Paulo State, Brazil, for the period of 2004–2005.



Figs. 2–4. Cambial status categories. (2) Very narrow cambial zone with only one initial cell (arrow) in a row of cells in a 3-year-old module, March 2004 collection, of *Annona coriacea*. Nacreous thickenings are apparent in the sieve-tubes (s) shown here. (3) Cambial activity has begun as evidenced by the recently formed, very narrow periclinal cell walls (arrows) in a 1-year-old module, October 2004 collection, of *Gochnatia barrosoae*. Sieve-tubes (s) are indicated. (4) Active cambial zone (cz) and differentiating cells on both sides are seen in a 3-year-old module, October 2004 collection, of *Piptocarpha rotundifolia*. Differentiating vessels (*) are indicated. X = secondary xylem. P = secondary phloem.

delay or advance in the timing of the cambial activity depending on the age module or species (Table 1). However, in 2005, at the end of the dry season (collection of September, 35.1 mm of rainfall), some branch modules in *A. coriacea*, *P. rotundifolia*, *D. hispida*, *B. virgilioides*, *M. villosum*, *B. verbascifolia*, and *R. montana* had already initiated cambial activity (Table 1).

The cambium was already dormant in most species by the time of collection in March 2004 (122 mm of rainfall, Fig. 1), and was ending activity at the time of collection in February 2005 (66.6 mm of rainfall, Fig. 1), even though both these collections were in the rainy season (Table 1). Dormancy of the vascular cambium continued throughout the dry season both years (collection of June 2004; 34.5 mm of rainfall, and June 2005; 51.2 mm of rainfall, Fig. 1) in all branch modules of the ten species (Table 1).

Positive correlations were found between cambial activity and day length, precipitation and temperature for the three age classes of modules grouped for all species (Table 2), evergreen taxa only (Table 3) and deciduous taxa (Table 4). Considering the age modules as separate classes, day length, precipitation and temperature were positively correlated to cambial activity for all species (Table 2). For evergreen taxa, day length was positively correlated to cambial activity in the three age classes (Table 3), and temperature and precipitation were positively correlated to cambial activity in 2- and 3- year-old modules (Table 3). For deciduous taxa, day length was positively correlated to cambial activity in the three age classes (Table 4), temperature was positively correlated to cambial activity only for 3-year-old modules (Table 4), and precipitation was positively correlated to cambial activity only for 1-year-old modules (Table 4) (see also Supplementary material).

In relation to leaf phenology, budding was positively correlated to cambial activity for all three age classes (Table 2), evergreen (Table 3), and deciduous (Table 4). Considering the branch ages separately, budding was positively correlated to the cambial activity in the three age classes for all species, and evergreen taxa (Tables 2 and 3). For deciduous species, budding was positively correlated to the cambial activity in 1-year-old modules, and negatively correlated in 3-year-old modules (Table 4) (see also Supplementary material).

Comparing the three models for each circumstance analyzed, the model with the lowest Akaike's information Criterion (AIC)

Table 2
Results of the binomial generalized linear mixed-effect models testing the effects of environmental factors (maximum temperature, precipitation, daylength) and budding on the cambial activity of 1-, 2-, 3-year-old branches of all species. MOD = model. AIC = Akaike's information criterion. DF = degrees of freedom. σ = standard deviation. β = unstandardized coefficient. SE = standard error.

			1-, 2-, 3-yr-old branches			1-yr-old branches			2-yr-old branches			3-yr-old branches		
			MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3
	AIC		140.7021	211.6981	197.9368	128.9894	178.2245	178.6252	119.8959	164.5001	149.0045	116.8213	167.0271	138.7234
	DF		4	5	5	5	5	5	5	5	5	4	4	5
Random effects	Species/individual	σ	0.0000	0.0013	0.0000	0.6213	0.0000	0.0000	1.2480	0.0000	0.0004	0.0006	0.0000	0.0000
	Species	σ	0.9047	0.6972	0.9227	0.9716	0.6884	0.7879	2.4880	1.3090	1.7492	1.9637	1.2110	2.1990
Fixed effects	(Intercept)	β	−38.0423	−2.6863	−12.6394	−37.4384	−3.0460	−7.2422	−55.1038	−3.4414	−15.6541	−38.5796	−3.1708	−20.7750
		SE	5.6666	0.5171	2.5245	9.0125	0.5857	2.4687	16.3288	0.7435	4.5911	7.5189	0.6813	4.2391
		z	−6.7130	−5.1950	−5.0070	−4.1540	−5.2010	−2.9340	−3.3750	−4.6290	−4.3590	−5.1310	−4.6540	−4.9010
		p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0034	0.0007	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Max temperature (°C)	β			0.4307			0.1966			0.4930			0.6953
		SE			0.0931			0.0926			0.1262			0.1481
		z			4.6250			2.1230			3.9070			4.6950
		p			<0.0001			0.0337			<0.0001			<0.0001
	Precipitation (mm)	β		0.0180			0.0127			0.0140			0.0207	
		SE		0.0051			0.0056			0.0061			0.0061	
		z		3.5320			2.2620			2.3080			3.4160	
		p		0.0004			0.0237			0.0210			0.0006	
	Daylength (h)	β	3.0351			2.8434			4.1677			2.9658		
		SE	0.4538			0.6937			1.2413			0.5878		
		z	6.6890			4.0990			3.3580			5.0460		
		p	<0.0001			<0.0001			0.0008			<0.0001		
	Budding	β		0.5901	0.7933	0.8693	0.9057	1.0032	0.9971	0.8351	1.1478			0.7550
		SE		0.1664	0.1848	0.3044	0.1988	0.2032	0.4238	0.2047	0.2481			0.2493
		z		3.5450	4.2940	2.8560	4.5560	4.9360	2.3530	4.0800	4.6270			3.0290
		p		0.0004	<0.0001	0.0043	<0.0001	<0.0001	0.0186	<0.0001	<0.0001			0.0025

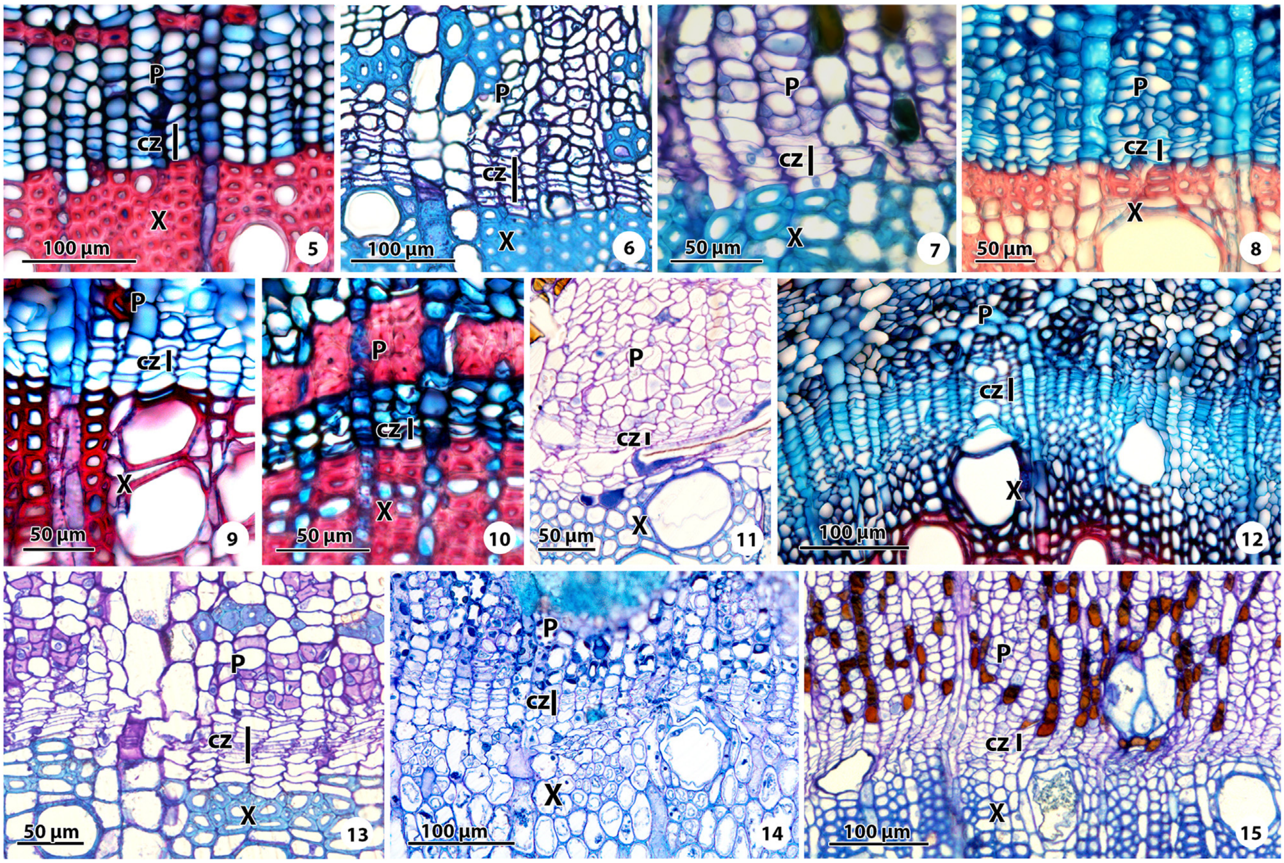
Table 3
Results of the binomial generalized linear mixed-effect models testing the effects of environmental factors (maximum temperature, precipitation, daylength) and budding on the cambial activity of 1-, 2-, 3-year-old branches of evergreen species. MOD = model. AIC = Akaike's information criterion. DF = degrees of freedom. σ = standard deviation. β = unstandardized coefficient. SE = standard error.

			1-, 2-, 3-yr-old branches			1-yr-old branches			2-yr-old branches			3-yr-old branches		
			MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3
	AIC		119.671	172.152	163.718	112.292	147.371		95.924	133.165	125.246	92.570	129.139	111.604
	DF		4	5	5	5	4		5	5	5	4	4	5
Random	Species/individual	σ	0.1333	0.0005	0.0000	0.7361	0.0000		1.1470	0.0014	0.2176	0.0000	0.0000	0.0000
effects	Species	σ	0.9944	0.8593	1.0310	0.9795	0.8459		2.3090	1.3520	1.7425	2.1930	1.4080	2.4170
Fixed effects	(Intercept)	β	−35.8520	−2.6495	−11.0792	−33.3376	−2.2558		−46.980	−3.5133	−13.4620	−37.314	−3.3113	−18.8474
		SE	7.1090	0.6181	2.6680	8.7900	0.5047		14.7200	0.8708	4.0502	8.3346	0.0260	4.5350
		z	−5.0430	−4.2870	−4.1530	−3.7930	−4.4700		−3.1910	−4.0350	−3.3240	−4.4770	−1266.3	−4.1560
		p	<0.0001	<0.0001	<0.0001	0.0001	<0.0001		0.0014	<0.0001	0.0009	<0.0001	<0.0001	<0.0001
	Max temperature (°C)	β			0.3689						0.4111			0.6149
		SE			0.0990						0.1395			0.1575
		z			3.7270						2.9480			3.9040
		p			0.0002						0.0032			<0.0001
	Precipitation (mm)	β		0.0158						0.0142			0.0203	
		SE		0.0057						0.0069			0.0050	
		z		2.7540						2.0640			4.1000	
		p		0.0059						0.0390			<0.0001	
	Daylength (h)	β	2.8620			2.5202			3.5321			2.8526		
		SE	0.5690			0.6782			1.1147			0.6503		
		z	5.0290			3.7160			3.1690			4.386		
		p	<0.0001			0.0002			0.0015			<0.0001		
	Budding	β		0.6601	0.7840	0.8469	1.0105		0.9523	0.8745	1.0974			0.7219
		SE		0.1945	0.2083	0.3330	0.2285		0.4258	0.2334	0.3066			0.2805
		z		3.3950	3.7640	2.5430	4.4220		2.2370	3.7470	3.5790			2.5740
		p		0.0007	0.0002	0.0110	<0.0001		0.0253	0.0002	0.0004			0.0101

Table 4

Results of the binomial generalized linear mixed-effect models testing the effects of environmental factors (maximum temperature, precipitation, daylength) and budding on the cambial activity of 1-, 2-, 3-year-old branches of deciduous species. MOD = model. AIC = Akaike's information criterion. DF = degrees of freedom. σ = standard deviation. β = unstandardized coefficient. SE = standard error.

			1-, 2-, 3-yr-old branches			1-yr-old branches			2-yr-old branches			3-yr-old branches		
			MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3	MOD.1	MOD.2	MOD.3
	AIC		26.0810	46.5818	37.8377	26.0810	39.2363	44.5221	26.0810			23.7099		36.6404
	DF		4	4	5	4	5	4	4			5		4
Random effects	Species/individual	σ	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			57.4400		0.0000
	Species	σ	0.0000	0.0000	0.0000	0.0000	0.0000	0.3071	0.0000			30.7700		0.6129
Fixed effects	(Intercept)	β	−52.225	−3.0508	−31.2761	−52.225	−3.9912	−2.0051	−52.225			−447.307		−18.4909
		SE	15.989	1.0125	13.8214	15.989	1.3195	0.6632	15.989			57.927		6.3783
		z	−3.266	−3.013	−2.2630	−3.266	−3.025	−3.0230	−3.266			−7.722		−2.8990
		p	0.0011	0.0026	0.0236	0.0011	0.0025	0.0025	0.0011			<0.0001		0.0037
	Max temperature (°C)	β			1.1086									0.6663
		SE			0.4903									0.2379
		z			2.2610									2.8010
		p			0.0238									0.0051
	Precipitation (mm)	β		0.0310			0.0262							
		SE		0.0118			0.0133							
		z		2.6280			2.0000							
		p		0.0086			0.0455							
	Daylength (h)	β	4.149			4.149			4.149			36.231		
		SE	1.264			1.264			1.264			4.701		
		z	3.283			3.283			3.283			7.707		
		p	0.0010			0.0010			0.0010			<0.0001		
	Budding	β			1.5754		0.8174	0.8998				−4.147		
		SE			0.7337		0.3669	0.3726				1.609		
		z			2.1470		2.228	2.4150				−2.578		
		p			0.0318		0.0259	0.0157				0.0010		



Figs. 5–15. Seasonal cambial activity. (5–10) Dormant cambium. (5) Two-year-old module, June 2005 collection, *Gochnatia barrosoae*. (6) Three-year-old module, June 2004 collection, *Piptocarpha rotundifolia*. (7) Two-year-old module, March 2004 collection, *Diospyros hispida*. (8) Three-year-old module, February 2005 collection, *Bowdichia virgilioides*. (9) Two-year-old module, June 2005 collection, *Byrsonima verbascifolia*. (10) Three-year-old module, June 2004 collection, *Pouteria torta*. (11–15) Active cambium. (11) One-year-old module, February 2005 collection, *Roupala montana*. (12) One-year-old module, October 2004 collection, *Machaerium villosum*. (13) One-year-old module, October 2004 collection, *Annona coriacea*. (14) One-year-old module, February 2005 collection, *Leptolobium elegans*. (15) One-year-old module, February 2005 collection, *Diospyros hispida*. cz = cambial zone. X = secondary xylem. P = secondary phloem.

value was the model one (MOD. 1) which correlates day length to cambial activity for every circumstance, and correlates budding to cambial activity for 1- and 2-year-old modules in all species and evergreen taxa, and for 3-year-old modules in deciduous taxa.

4. Discussion

In this study, we investigated cambial activity in branch modules of different ages (1-, 2-, and 3-year-old) of ten species from the cerrado *sensu stricto* by an integrative approach relating cambial activity with climate factors and plant attributes in order to look for growth patterns for this ecosystem. Initially we analyzed the cambial zone of the three branch modules using histological slides with focus on the cambial activity. Then, we discussed the beginning and ending of the cambial activity with plant attributes (leaf phenology and habit) and climate factors (temperature, rainfall, day length).

We found that there is a distinct seasonality to cambial activity in 1-, 2- and 3-year-old modules with a relatively long dormancy period, lasting up to 7 months. This result differs appreciably from conventional wisdom that for many tropical species there is continuous cambial activity in most months of the year, or even throughout the year (Fahn, 1990; Iqbal, 1990).

It is noticeable that in the studied cerrado species, regardless of plant habit, the onset of cambial activity began during the dry season when the rainfall was 35.1 mm (September 2005) and the rainfall of the three prior months was less than 60 mm. This result conflicts with Worbes' (1995) statement that an annual dry season lasting from 2 to 3 months with a rainfall of less than 60 mm induces cambial dormancy; in these species, cambial activity begins in exactly those conditions. It is important to emphasize that most of the cambial activity studies has been conducted in main stems of forest trees, so Worbes' statement may not be generally applicable to branches of shrubs and small trees from cerrado *sensu stricto*, a savanna-like ecosystem. For our species, it is also noticeable that cambium was already dormant prior to the end of the rainy season (rainfall was 122.3 mm in March 2004). This finding differs from the results of other studies in the main stems of tropical species in which the cambial activity is concurrent with the rainy season (Dave and Rao, 1982b; Rajput and Rao, 2001; Tomazello-Filho and Cardoso, 1999; Rao and Rajput, 1999, 2000, 2001; Aref et al., 2014). A weak relationship of radial growth and monthly rainfall was observed for savanna species studied by Rossatto et al. (2009) and according to the authors, the lack of stem growth and leaf production at a time of year with a full canopy of actively-photosynthesizing leaves suggests that assimilate was being diverted to carbohydrate stores and/or root growth. This fact allows species to mitigate the effects of seasonal changes in water availability (Franco et al., 2005) and resprout after fire (Hoffmann and Moreira, 2002; Hoffmann et al., 2003).

Our results demonstrate that, in general, cambial activity (around 5 months) occurred when the accumulated rainfall values were the highest (around 66% of annual precipitation considering September as the beginning of the period, when the first rains fell after the dry season, Fig. 1). Trouet et al. (2012) studying *Brachystegia spiciformis*, a savanna species of south-central Africa, also observed a short period of cambial activity (about 4 months), and that it was not simultaneous with the beginning and ending of the rainy season, however it corresponded to the core of the rainy season. Differently to our findings, Trouet et al. (2012) found that cambial activity began later in the rainy season when 10–30% of the annual precipitation had occurred, and ceased when 75–82% of the total annual rainfall had been reached. In the branches of the species studied here, cambial activity began before the rainy season when less than 10% of the annual precipitation had occurred, and ceased when around 66% of the total annual rainfall had been reached. Although the onset and end of cambial activity were not synchronized with the beginning and ending of the rainy season, the intensity of cambial divisions to produce the secondary vascular tissues, which is highly variable, might be influenced mainly by precipitation, among other climatic factors.

In our results the maximum temperature were correlated with cambial activity when combining age classes in all species, evergreen and deciduous taxa, reinforcing what was found by Yáñez-Espinosa et al. (2006, 2010) for branches from five species of subtropical forests with seasonal drought in Mexico. These results confirm that temperature is an important factor for the resumption of cambial activity in tropical species (Waisel and Fahn, 1965; Rao and Dave, 1981; Dave and Rao, 1982a,b; Ajmal and Iqbal, 1987; Paliwal and Paliwal, 1990; Rao and Rajput, 1999; Venugopal and Liangkuwang, 2007). Temperature is required to induce new buds and foliage and thus induce cambial activity by the presence of growth hormones (Rao and Rajput, 1999).

Although in our results the maximum temperature, precipitation and day length were correlated with cambial activity in all module ages (1-, 2-, 3-year-old), day length is the environmental factor that better explain cambial activity considering the best model (lowest AIC value).

Several authors mention the relationship between budding and cambial activity (see Dave and Rao, 1982a,b; Venugopal and Krishnamurthy, 1987; Aref et al., 2014). In our study, this relationship was clearly observed for all species and evergreens. For 2-year-old modules of deciduous taxa there was no relation between budding and cambial activity, and for 3-year-old modules of deciduous taxa this relation was negative. Taking into account that: (1) the major sources of indole-3-acetic acid (IAA) are the buds in development and the young shoot (Scarpella and Meijer, 2004; Uggla et al., 1998 *apud* Sorce et al., 2013); (2) IAA is the main hormone involved in the regulation of cambial activity (Sorce et al., 2013); (3) IAA moves polarly and continuously from the leaves to the roots (Aloni, 2013); and (4) there is a general decrease in IAA concentrations basipetally in the stem (Aloni, 2001); our results suggest that there is a relation between newly produced IAA and cambial activity closer to the stem apex, mainly for deciduous taxa.

The relations among budding, day length, and the onset of cambial activity in the species from cerrado, both evergreen and deciduous, was evidenced in this study. The importance of the seasonal variation in day length, and not the climatic seasonality to break dormancy of buds for cerrado species was reported by Rivera et al. (2002). According to the authors, an increase of 30 min or less in day length induced sprouting, and the critical day length for the induction of bud break ranges from 11.5 to 12.5 h in the subtropics and is ~12 h at low latitudes. Because day length varies in a predictable annual cycle constant through Eras, it dictates many biological cycles in plants, presumably the cambial activity period might be one of them.

In summary, for the ten study species from cerrado *sensu stricto*, regardless of plant habit and leaf shedding patterns, cambial activity was not simultaneous with the beginning and ending of the rainy season; however, it corresponded to the core of the rainy season (when at least 66% of the annual precipitation had been reached). The onset of cambial activity occurred before the rainy season and end of cambial activity occurred during the rainy season. Our data suggest that, more than rainfall, day length seems to control onset and cessation of cambial activity in branches. Studies on cambial activity comparing main stem and branches in the same species are already in progress and will provide a better understanding of the cambial performance on cerrado plants. Our results are helpful to allow us to understand the growth dynamics of Brazilian savanna (cerrado) species compared to other vegetation types, implying a possible influence of global climate change on the increase of the radial growth rates.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.flora.2016.04.008.

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