The corewood of 25-year-old *Hevea brasiliensis* from two rubber plantations has high starch content

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ABSTRACT

In Brazil after 25 to 30 years of rubber production, when yield starts to drop, rubber trees are felled and destined for firewood and charcoal, despite the good mechanical properties and workability of the wood, and relatively low production costs. Wood with low starch content could be destined for the production of higher added-value products with potential to spare deforestation of many native forest species, but in rubberwood starch increases palatability by wood borers and accelerates fungal degradation, thus compromising wood durability and the quality of timber. The aim of this study is to determine whether removal of the outer part of wood or varying the season of logging would result in wood with lower starch content. We measured the content of starch using enzymatic hydrolysis, the radial distribution of starch grains by light microscopy, and the corresponding seasonal variation of starch in 25-year-old felled trees. Rubberwood had large amount of starch in its entire trunk, increasing from the inner to the outer region, before decreasing in the outermost sapwood. Starch content was lower in summer, although higher than in other timber species. After relating our data to a comprehensive bibliographic survey of starch quantification in rubberwood, we concluded that there are no technological arguments to destine the inner part of rubber tree trunks to the production of higher value products.

Key words: starch content, starch grains, rubberwood, tyloses

DECLARATIONS

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Conflicts of interest

The authors have no conflicts of interest.

Availability of data and material

The dataset is available upon request.

Code availability

We did not develop new code in this study

Author contribution

SGC and AWB designed the study; SGC performed analysis with contribution of CB, TPRS, SAR, ML, CRM; SGC, CRM, AWB, SAR analysed the data; SGC, AWB, CB, CRM wrote the manuscript; AWB is the corresponding author. All authors have read and agreed to this version of the manuscript.

KEY MESSAGE

The corewood of 25-year-old *H. brasiliensis* has lower starch content than the sapwood, but this content is two or three folds higher than the content commonly observed in the heartwood of timber species.

INTRODUCTION

Hevea brasiliensis (Willd. ex A.Juss.) Müll.Arg., commonly known as rubber tree, has high economic importance as the main source of natural rubber, a product with increasing global demand and multiple applications in the industry (Martin and Arruda 1993). Rubber tree originates from the tropical rainforests of the Amazon in South America, warm and moist throughout the year. Outside its native regions, it may have a deciduous behaviour which may become more pronounced especially where there is a dry season (Cardoso et al. 1989; Kumagai et al.

2015). In Brazil, plantations of rubber trees reach 218,307 ha (IBA 2019); the clones RRIM 600 and GT-1, are the most planted and productive. After 25 to 30 years, when rubber production starts declining, trees are replaced and the wood is traditionally destined to low added value products, like, for example, pallets and fuel (Eufrade Júnior et al. 2015). Rubberwood presents favourable physical and mechanical properties (Eufrade Júnior et al. 2015), good workability, and low production costs (Killman and Hong 2000; Balsiger et al. 2000; Hashim et al. 2005, Teoh et al. 2011) and can be used in small structures, lightweight construction, indoor building components, panels (Faria et al. 2020a), wooden toys (Lim et al. 2003) and furniture. Inexpensive rubberwood furniture is well-perceived in Southeast Asia (Ratnasingam et al. 2007). In Malaysia and Thailand, where there is strict control on forest logging and support for rubberwood production, in 2007 more than 35% and 60%, respectively, of the total exported wood products (furniture, sawn wood, and logs) were made with rubberwood (Shigematsu et al. 2011). Use of rubberwood for timber has therefore the potential to spare deforestation of many native forest species (Eufrade Júnior et al. 2015; Severo et al. 2016).

A key factor limiting the utilization of rubberwood is durability (Milingliang and Zhijuan 2008; Severo et al. 2016). Untreated rubberwood is moderate resistant to decay – class 3 of ASTM D2017, which means average weight losses of 25 to 44% [please indicate for non experts over what period and in what conditions the weight is lost] (ASTM 2017; Rodrigues et al. 2018; Uyup et al., 2019). Wong et al. (2005), based on a regional classification used by the Malaysian market, reported class 4 (losses of 11 to 30% [please indicate for non experts over what period and in what conditions the loss occurs]- wood has less than two years life in tropical climates). The ephemeral life of rubberwood is due to the high starch content, which makes wood appetible to fungi and insects (Hong and Sim 1994; Hong 1995; Silpi et al. 2007; Chantuma et al. 2009; Servolo Filho 2013). In fact, starch contents higher than 8g/100g have been reported in rubberwood (Kadir and Sudin 1989; Hong and Sim 1994; Hong 1995; Santana and Eiras 1999; Killmann and Hong 2000; Silpi et al. 2007; Milingliang and Zhijuan 2008; Tamolang 2008; Chantuma et al. 2009; Teoh et al. 2011), while in timber species starch content ranges from 1g/100g to 3g/100g (Santana and Eiras 1999).

Wood is normally distinguished into an outer conducting portion containing living parenchyma cells called sapwood, and an inner portion, called heartwood, where parenchyma cells are dead, and the vessels are clogged by the deposition of tannins, resins, phenols, and terpenes. These compounds, sometimes collectively referred to as extractive, help make heartwood more resistant to attack by insects and decay but also tend to give this inner portion of the stem a distinctive darker colour. Starch is commonly present in the sapwood, and some authors reported the absence or only traces of starch in the heartwood of *Robinia pseudoacacia* (Magel et al. 1994), *Juglans nigra* (Dehon et al. 2002), *Pinus sylvestris* (Bergström 2003), *Larix kaempferi* (Nakada and Fukatsu 2012) and *Tectona grandis* (Niamké et al. 2011, 2018), contributing to their durability. Rubberwood is difficult to differentiate by colour (Killmann and Hong 2000; Edwin and Ashraf 2006; Teoh et al 2011), but the inner part of the trunk, called corewood, does not conduct sap because vessels are occluded by outgrowths of parenchyma cells, called tyloses (Evert 2006, Spicer 2016). In corewood there is an external transition zone, where some parenchyma cells are alive, to the internal heartwood, which may only occur in older trees. If a gradient of starch content is present in rubberwood, the inner part of the trunk could be used to derive timber, while the remainder could be used for low added value products or energy generation. Furthermore, deciduous trees present an abrupt starch decrease after re-foliation and flowering (Lacointe et al. 1993; Witt and Sauter 1994; Barbaroux et al. 2003; Silpi et al. 2007), and if seasonality in starch content is also present in rubber trees, it could be harnessed to increase the quality of timber.

We set out to investigate whether the seasonal and spatial variation in starch content in rubberwood can be exploited to obtain timber with lower starch content. We measured the radial distribution of starch in the main trunk of *Hevea brasiliensis* in two seasons (winter and summer).

MATERIAL AND METHODS

Sites

Trees were growing with spacing of 7m between rows and 3m between plants in two experimental plantations in Brazil:

- Mococa (APTA *Regional Nordeste Paulista*) 21° 28' S and 47° 01' W, average altitude of 621m, on Red Argisol of clayey texture, although it has high capacity of water storage, it has physical limitations, namely low depth and presence of gravel or pebbles on the surface (IAC/APTA 2015). The climate classification is Aw (Köppen-Geiger), with annual precipitation 1168mm (Climate-Data.Org. 2021);
- Ribeirao Preto (APTA *Regional Centro Oeste*) 21° 12' S and 47° 52' W, average altitude of 534m, on Red Latosol with clayey texture is fertile and has good physical properties (IAC/APTA 2015). The climate classification is Aw (Köppen-Geiger) with annual precipitation of 1384mm (Climate-Data.Org. 2021).

The climate data of the seasons, representative of the last 10 years in each of the sites are shown in Table S1. Both experimental sites were similar in climatic conditions in their respective seasons (Table S2).

Sampling

Eight 25-year-old trees (clones RRIM 600) were randomly felled at each site in August 2016 (winter) and February 2017 (summer) totalling 32 trees (8 trees × 2 sites × 2 seasons).

One 8 cm-thick disk was cut 30 cm above the ground from each tree for starch quantification every one-fifth long the larger radius of the tree (Fig. 1A). Specimens varied in width (W) from 2.60 cm to 4.40 cm (Fig. 1A).

For microscopic observation of tyloses and starch grains distribution along the radius, a disk was sampled just below the other disk, in a random tree at each site and at each season, obtaining samples every 1cm along the radius (Fig. 1B).

Starch content

Samples were oven dried at 70ºC and milled into a fine powder with no evident fibre structure. An internal reference standard (IRS) was prepared by pooling small aliquots from all wood samples and thoroughly mixing repeatedly (Bellasio et al. 2014). We analysed the IRS for several days until the reading stabilized before measuring samples.

Starch was quantified following procedure of Bellasio et al. (2014), optimized for the analysis of starch in wood. Briefly, starch was hydrolysed with α-amylase (*Bacillus licheniformis* E-BLAAM, Megazyme, Ireland) and then with high purity amyloglucosidase (*Aspergillus niger* E-AMGDF, Megazyme, Ireland). The resulting glucose was assayed through a coupled enzymatic reaction of *o*-dianisidine (PGO kit, Sigma, St Louis, USA), spectrophotometrically quantified.

Starch grains and tyloses in the wood

We cut 15 to 20 µm thick transverse sections in sliding microtome and observed using a light microscopy (Axioscop 40) with a camera Axiocam MRC – (ZEISS, Germany). Starch grains were revealed by embedding the wood sections in an aqueous Lugol's iodine solution (I_2KI) for 2 min (Wargo 1975). Regions where we observed tyloses plus starch grains were classified as corewood, and those without tyloses were classified as sapwood.

Statistical analysis

The mixed generalized linear model (GLMM) with gamma probability distribution and logarithmic link function (Nelder and Wedderburn 1972; Bolker 2015) was used to evaluate the effects of the radial position, site and season on the starch content, considering the variability of repeated measures of trees along their radius. The site, season and radial position covariates were included in the model as a fixed effect and the individual tree as a random effect, and were followed by a Tukey post-hoc multiple comparison test with 5% probability. The comparison between climatic data of the sites, measured annually in the winter and summer seasons in the last 10 years, was performed using the Mann-Whitney test and results expressed as median (range). All analyses were performed in software R v3.5.2 (R CORE TEAM 2019), using the R-package 'lme4' to develop the GLMM (Bates et al. 2013) and R-package "emmeans" to the post-hoc tests (Lenth 2020).

RESULTS

Starch content

Starch content ranged from 6 to 17g/100g (Table 1), showing a well-defined radial distribution, increasing from the centre to 80% of the radius, followed by a decrease closer to bark, for both collection sites and seasons. The average starch content was higher in winter than in summer along the entire radius for the two sampling sites and lower in Mococa than in Ribeirao Preto (Fig. 2).

Microscopic analysis of the samples

Figure 3 shows cross section micrographs of rubberwood where starch grains were stained with lugol solution ordered from the innermost (Panel A) to the outermost (Panel I). Starch grains appear in all sections.

Corewood differentiated from the outermost region (sapwood) by the presence of tyloses plus starch grains. The width of corewood ranged from a minimum of 3 cm wood harvested from Mococa to 7 cm in wood harvested from Ribeirao Preto (Table 2). Interestingly, trunks from Ribeirao Preto had smaller diameter than those from Mococa (27 and 36 cm, respectively) but a higher proportion of corewood (48% and 22%, respectively).

DISCUSSION

Starch grains stored in the parenchyma cells of the secondary xylem have an important role in the tree's functioning, as these can be hydrolysed into soluble sugars to repair cavitation, translocated to heterotrophic organs of the tree that need these sugars, and can be used when photosynthesis does not supply the carbon requirements for maintenance and growth (Glerum 1980; Kozlowski 1992; Lacointe et al. 1993, 1995; Witt and Sauter 1994;

Barbaroux and Bréda 2002; Hoch et al. 2003; Silpi et al. 2007; Plavcová and Jansen 2015). In particular, in the deciduous species, including *H. brasiliensis* when behaving deciduously, starch is the unique carbohydrate source during the sprouting period (Lacointe et al. 1993; Barbaroux and Bréda 2002). Starch severely limits wood durability, making it appetible to fungi and insects (Hong and Sim 1994; Hong 1995; Silpi et al. 2007; Chantuma et al. 2009; Servolo Filho 2013). Durability was repeatedly identified as the main limit to the broad application of rubberwood (Hong et al., 1999, e.g.). Nevertheless, rubberwood presents favourable physical and mechanical properties, shown in our previous studies (Eufrade Júnior et al. 2015), good workability and low production costs. Unfortunately, in almost all previous research, durability of rubberwood was evaluated in qualitative terms (HONG et al., 1999, e.g.), comparing its performance with other timber species, without setting a target of required durability for timber exploitation. On the other hand, standard studies of rubberwood durability are quite recent, reporting the class of durability, but not evaluating the starch content (Rodrigues et al. 2018, e.g.).

We set out to investigate whether inner regions of the trunk of rubberwood have lower starch content, which could justify lamination (peeling) of sapwood and technological exploitation of corewood, for instance, in the production of preservative-free high added-value products. Our study is the only one investigating the radial distribution of starch in rubberwood. We found high contents of starch in all regions of rubberwood (6.2 – 17.5 g/100g, Table 1). Starch content decreased towards the centre of the trunk (Table 1), but even the minimum starch content observed in the innermost part was two or three folds higher than the content commonly observed in the heartwood of timber species (1g/100g −3g/100g; Santana and Eiras 1999), suggesting that the inner part of corewood was not yet differentiated into heartwood. In other species the starch content in heartwood is generally negligible (e.g. *Pinus sylvestris* - Bergström 2003, *Robinia pseudoacacia* - Magel et al. 1994, *Juglans nigra* - Dehon et al. 2002, *Larix kaempferi* - Nakada and Fukatsu 2012, *Tectona grandis* - Niamké et al. 2011, 2018) We conducted a comprehensive bibliographic survey of previous studies investigating starch content in rubberwood (Table 3). True heartwood apparently does not form in rubber tree plantations. Although we cannot exclude that true heartwood may eventually form in older trees in the wild we did not find any study reporting its characteristics and starch content.

Several factors influence starch content, like the age of the plantation, the phenology, the season, and site characteristics – soil and climate. Silpi et al. (2007) and Chantuma et al. (2009) observed low starch content in 8 to 9-year-old juvenile plantations. Young plants have higher growth rates with more pronounced radial growth, especially in the summer season, i.e., rainy season in a tropical environment, when trees have leaves and fruits (Rodrigo 2007). Chantuma et al. (2009) reported radial growth in juvenile plantations even in the dry season (conditions DS+RAGR [can you explain for non experts please], Table 3), differently from what we observed in mature plantation. Higher growth rate and latex production divert carbohydrates for biomass and respiration, possibly competing with allocation to starch (Lacointe et al. 1993; Witt and Sauter 1994; Barbaroux et al. 2003). Sapwood starch might be more readily converted into soluble sugars and mobilised than corewood starch (Magel et al. 1994, Sala et al. 2011). The increase of sapwood starch content in winter provides evidence that trees tend to adjust the amount of carbohydrate reserves to the lower needs for maintenance, growth, and reproduction. Perhaps, the lower carbon requirements for growth, production, and maintenance of mature trees can be satisfied by the outermost sapwood. Therefore, starch would not be a sink for carbon overspill, but rather an 'insurance' against adverse conditions. However, the reasons for maintaining high starch content in the corewood and invariant through the seasons are unknown.

Considering high starch contents observed along the entire radius of rubber trees trunks in all the situations evaluated, laminating would have no value to enhance durability; therefore, the usage of its wood for products that demand higher durability, would require prophylactic preservative treatments immediately after processing of logs (Teoh et al. 2011), and impregnation of chemicals after drying (Faria et al. 2020b), or use of chemical modification of wood without the use of biocides (Oldertrøen et al. 2016), heat treatments using hot oil (Umar et al. 2016) or steam (Bakar et al. 2013; Severo et al. 2016; Zhang et al. 2019) as heating media for rubberwood conditioning.

CONCLUSION

We conclude that rubber trees accumulate a large amount of starch in its wood, from the innermost to the outermost regions of sapwood. In summer, starch content is lower, although higher than in other timber species. The corewood has considerable starch content, commonly associated with low natural biological durability. There are no technological arguments to destine rubber corewood to timber.

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Figure captions

Fig. 1. Sampling scheme for starch quantification (A) and for microscopic observation of tyloses and starch grains (B).

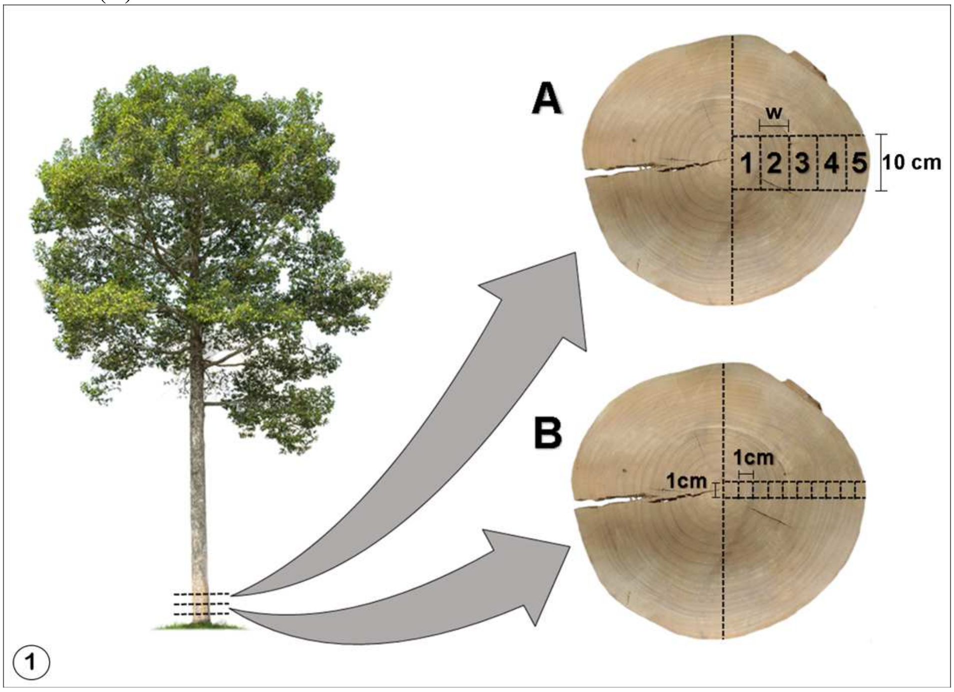

Fig. 2. Radial variation of starch content in rubberwood felled in summer and winter in two different sites (A, B), or, same data alternatively plotted in two seasons (C, D). In A and B, different lowercase letters represent significative differences between the samples for the same season and uppercase letters represent significative differences between the seasons for the same samples. In C and D, different lowercase letters represent significative differences between the samples for the same site and uppercase letters represent significative differences between the sites for the same samples.

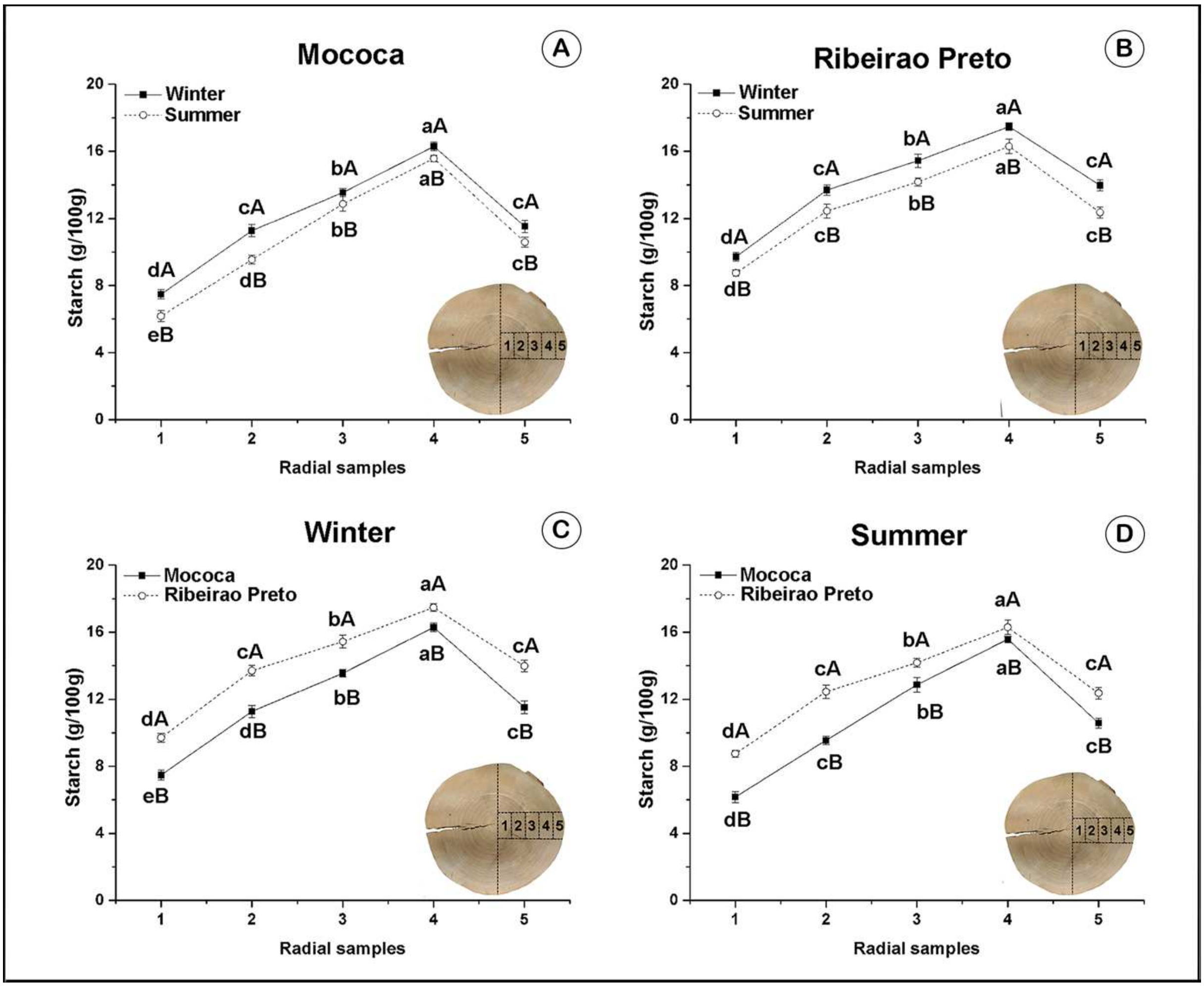

Fig. 3. Micrographs of cross sections collected every 2 cm of the trunk radius from A (closest to the pith) to I (closest to the cambial zone) of trees from the Mococa plantation felled in summer. Starch grains are the dark points (the presence of starch grains indicates living parenchyma cells) and tyloses are shown by arrows. All images are in the same magnification. Bar = 200 µm. Every second of the micrographs collected for each 1 cm of radius (Fig. 1B) is shown.

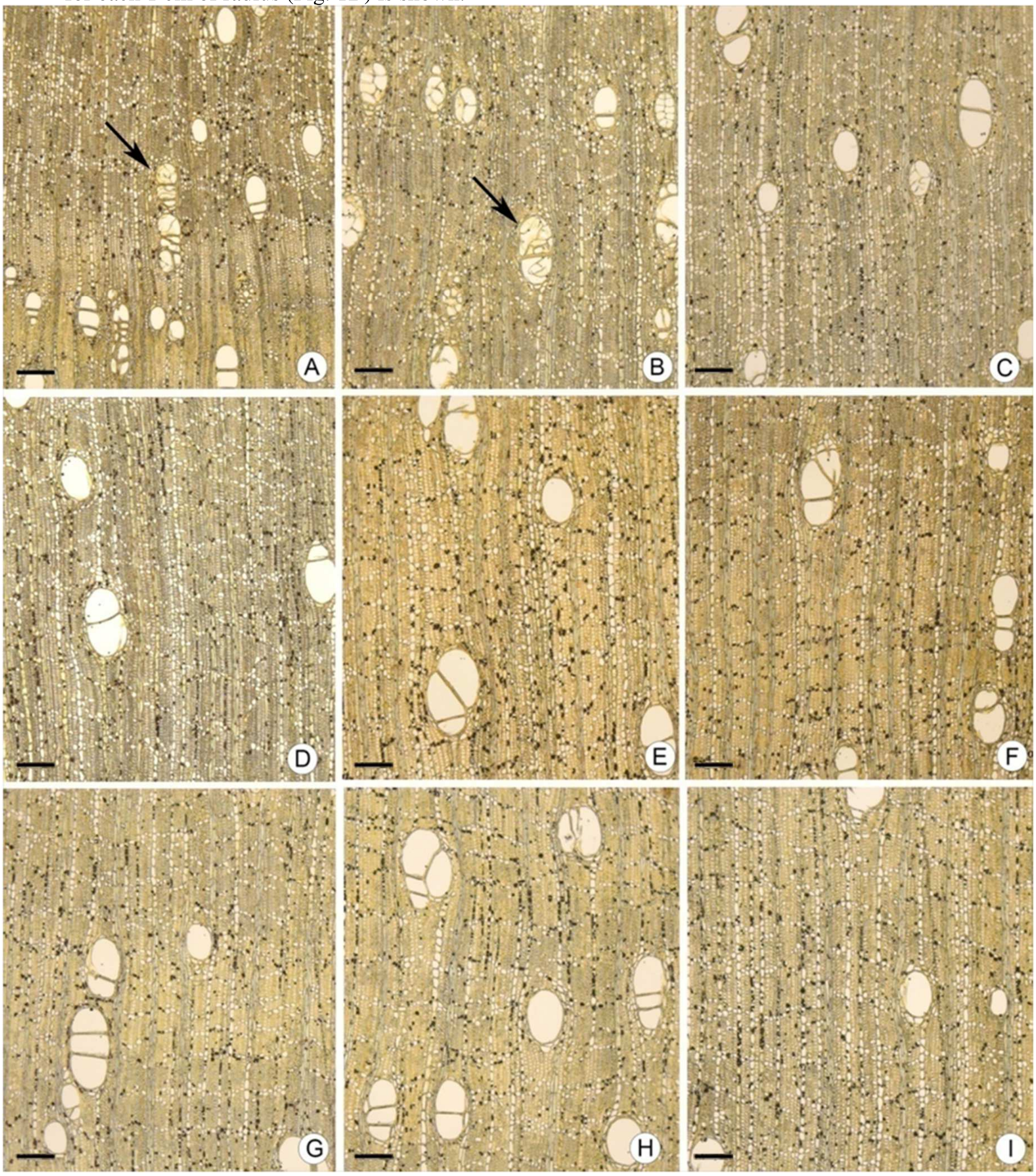

Table 1. Starch content (g/100g) every one fifth along the larger radius of the tree from pith (1) to bark (5). Mean ± Standard Deviation.

Site	Season	Radial samples				
		1	**2**	**3**	**4**	**5**
Mococa	Winter	7.5±0.28**d**	11.3±0.36**c**	13.6±0.23 **b**	16.3±0.27 **a**	11.5±0.38 **c**
	Summer	6.2±0.30 **e**	9.6±0.26 **d**	12.8±0.41 **b**	15.6±0.18 **a**	10.6±0.27 **c**
Ribeirao Preto	Winter	9.7±0.26 **d**	13.7±0.31 **c**	15.4±0.39 **b**	17.5±0.22 **a**	14.0±0.34 **c**
	Summer	8.7±0.19 **d**	12.5±0.40 **c**	14.3±0.31 **b**	16.3±0.41 **a**	12.4±0.33 **c**

Letters compare radial samples, according to site and season

Table 2. Mean radius (R), corewood (C) and sapwood (S) width and corewood percentage (C/R) of four *H. brasiliensis* 25-year-old trees from Mococa (summer and winter) and Ribeirao Preto (summer and winter).

Site	Season	Radius (R)	Corewood (C)	Sapwood (S)	C/R
		(cm)	(cm)	(cm)	(%)
Mococa	Winter	18.0	5.0	13.0	27.8
	Summer	18.0	3.0	15.0	16.7
Ribeirao Preto	Winter	13.0	7.0	6.0	53.8
	Summer	14.0	6.0	8.0	42.9

Table 3. Compilation on papers studying starch quantification in *H. brasiliensis* – information of sites, plantations and methodologies of experimental programs.

Study Characteristics	Authors			
	Present study	**Silpi et al. (2007)**	**Chantuma et al. (2009)**	**Servolo Filho (2013)**
Growing site	Brazil (Lat. 21° S)	Thailand (Lat ≈ 6° - 18° N)	Thailand (Lat ≈ 6° - 18° N)	Brazil (Lat. 21° S)
Age of plantation (years)	25	9	8	26
DBH (cm)	Mococa: 25.8 – Rib. Preto: 23.5 41.4	14.6 – 16.7	14.8 – 16.4	11.8– 22.9
Phenological Period and season (See abbreviations)	DS+WOL RS+WWL+FRU	DS+RAGR RS+RAGR DS+WOL+WORA DS+	DS+WOL+WORA DS+REGR RS+ WWL+RAGR DS+RAGR DS+WORA	WOL – leaves artificially removed WWL
Hydrolysis Methodology	α-amylase amyloglucosidase (Bellasio et al. 2014)	NaOH α-amiloglucosidase	NaOH α-amiloglucosidase (Boehringer 1984)	α-amylase amyloglucosidase (Rickard and Behn 1987)
Starch content (g/100g)	6.2 – 17.5	3.9 – 5.8	~ 5.5 – 6.5	5.5– 7.2
Abbreviations	**Season** DS - dry season RS - rainy season	**Phenol. Period –** WOL - without WWL - with leaves FRU - fruits	**Phenol. Period - growth** RAGR - with radial growth WORA - without radial growth REGR - regrowth	

Corewood of 25-year-old *Hevea brasiliensis* has high starch content

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Supplementary material

Table S1. Statistics of climate data for the sites in seasons (winter and summer) in 10-years periods prior to experimentation and climate data in the sampled years.

Site	Season/period or year	Tmax	Tmin	Tmean	Etp	Seasonal Accumulated rain
		(°C)			(mm)	
Mococa	**Win/2006-2015**					
	Mean	29.89	12.39	21.12	2.44	106.53
	std dev	0.61	0.75	0.41	0.05	91.58
	Win/2016	30.27	11.54	20.86	2.46	59.40
	Sum/2007-2016					
	Mean	31.59	18.75	24.90	4.44	631.52
	std dev	0.72	0.30	0.43	0.15	192.83
	Sum/2017	32.16	19.11	25.39	4.56	735.90
Ribeirao Preto	**Win/2006-2015**					
	Mean	30.24	12.64	21.34	2.50	90.55
	std dev	0.73	0.86	0.38	0.07	87.96
	Win/2016	30.46	12.28	21.03	2.44	55.80
	Sum/2007-2016					
	Mean	31.67	18.54	24.83	4.42	623.27
	std dev	0.61	0.35	0.39	0.13	152.89
	Sum/2017	31.96	18.88	25.20	4.45	433.00

Table S2. Comparison of climate data between sites by Mann-Whitney test, in seasons (winter and summer) in 10-year periods prior to experimentation.

Season/ Period	**Site**	**Statistics**	**Tmax**	**Tmin**	**Tmean**	**Etp**	**Accumulated rain**
			(°C)			(mm)	
Winter (2006-2015)	Mococa	Median	29.91	12.58	21.13	2.43	84.10
		[Min-Max]	29.07-30.80	11.18-13.30	20.32-21.70	2.37-2.51	29.40-343.70
	Ribeirao Preto	Median	29.97	12.56	21.44	2.51	70.15
		[Min-Max]	29.39-31.81	11.31-13.76	20.42-21.72	2.39-2.57	24.30-327.10
p-value			**0.393**	**0.631**	**0.140**	**0.045***	**0.436**
Summer (2007-2016)	Mococa	Median	31.32	18.72	24.65	4.36	625.30
		[Min-Max]	30.63-32.98	18.35-19.30	24.49-25-70	4.28-4.68	360.30-1049.80
	Ribeirao Preto	Median	31.57	18.54	24.83	4.40	641.85
		[Min-Max]	30.78-32.65	17.83-18.90	24.19-25.32	4.20-4.59	361.00-915.30
p-value			**0.579**	**0.190**	**0.796**	**0.970**	**0.971**

Note: (*) means statistically significant difference between sites ($p<0.05$)