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FENOLOGIA E DENDROCRONOLOGIA DE DUAS ESPÉCIES DE FABACEAE EM UMA ÁREA DE CERRADO NO SUDESTE DO BRASIL

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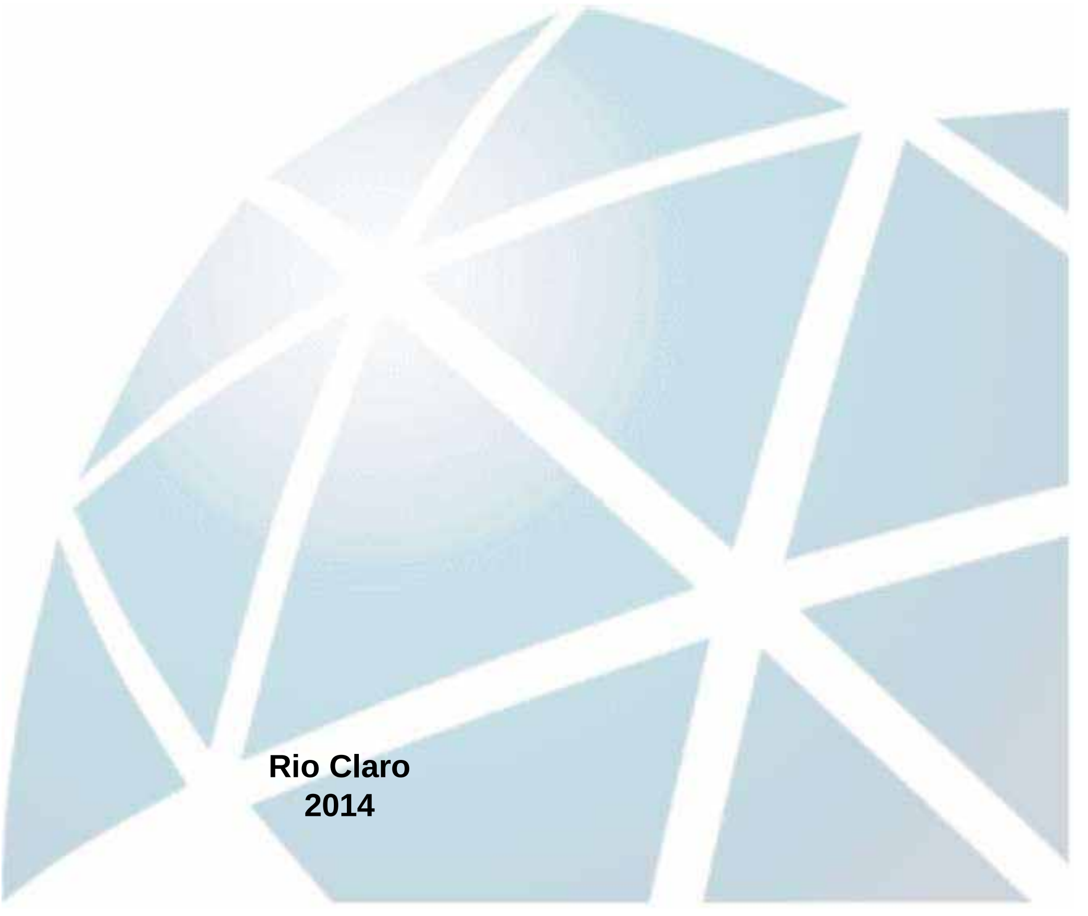

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FENOLOGIA E DENDROCRONOLOGIA DE DUAS ESPÉCIES DE FABACEAE EM UMA ÁREA DE CERRADO NO SUDESTE DO BRASIL

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“O correr da vida embrulha tudo.
A vida é assim: esquenta e esfria,
aperta e daí afrouxa,
sossega e depois desinquieta.
O que ela quer da gente é coragem.”

(João Guimarães Rosa)

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Apresentação

A dissertação foi estruturada em dois capítulos escritos no formato de artigo científico. Essa disposição auxiliará a banca a avaliar, criticar e dar sugestões já com a estrutura de artigo científico em mente.

O primeiro capítulo intitulado "Climate, phenology and growth rings of two Fabaceae species from the Brazilian cerrado" foi resultado de uma pesquisa que visou combinar a área da fenologia com a dendrocronologia. Para isso, toda a parte da pesquisa sobre a fenologia de ambas as espécies, *Anadenanthera peregrina var falcata* e *Pterodon pubescens*, foi realizada na UNESP – campus de Rio Claro - supervisionada pela Profa. Dra. Patrícia Morellato. Para que sua conexão com o padrão dos anéis de crescimento fosse possível, uma cooperação com o Prof. Dr. Gregório Ceccantini foi estabelecida e a segunda parte desta pesquisa foi realizada na USP – campus São Paulo, no laboratório de anatomia vegetal.

O segundo capítulo deste trabalho, intitulado "Growth rings, phenology and climate influence inferred by high-resolution stable isotopes: the case of *Pterodon pubescens*" foi resultado de uma pesquisa em união entre vários Institutos e pesquisadores. Visando a união da fenologia com os anéis de crescimento e seus isótopos estáveis de carbono, este trabalho foi majoritariamente desenvolvido na Universidade de Leeds, Inglaterra, sob a supervisão do Prof. Dr. Roel Brienen e no Instituto de Pesquisa GFZ – German Research Centre for Geosciences - em Potsdam, Alemanha, sob a supervisão do Prof. Gerhard Helle e com auxílio direto de Heiko Baschek, técnico do laboratório de isotopia do Instituto e principal ajuda do desenvolvimento desta análise.

SUMÁRIO

Resumo

A fenologia estuda a ocorrência de eventos biológicos repetitivos e sua relação principalmente com o clima, e também com os fatores bióticos como polinizadores e dispersores de sementes. A dendrocronologia estuda os anéis de crescimento das árvores e, com isso, a resposta da árvore as mudanças do clima, podendo também estimar a idade da árvore e revelar informações de sua ecologia. Além disso, a dendrocronologia possibilita a reconstrução do clima pela análise da anatomia do lenho, uma vez que a planta registra na forma de anéis sinais das mudanças de temperatura e da precipitação que afetaram seu crescimento e dormência. Dentro dos anéis de crescimento é possível entrar o padrão de isótopos tanto de carbono quanto de oxigênio, que podem revelar informações ecológicas importantes, como a influência da sazonalidade no crescimento da planta e sua fenologia, bem como a fonte de uso da água, sua sazonalidade sob influência do clima e suas mudanças. Neste sentido, os anéis cíclicos e seus isótopos de carbono e oxigênio são respostas anatômicas e fisiológicas que, se relacionadas a uma determinada fenofase reprodutiva ou vegetativa, podem permitir uma reconstrução fenológica e uma avaliação dos fatores climáticos influenciando esses padrões reconstruídos, com implicações ecológicas, no entendimento dos efeitos de mudanças climáticas nas plantas. Este trabalho teve como objetivo (1) analisar oito anos de fenologia de Fabaceae (*Anadenanthera peregina* var *falcata* e *Pterodon pubescens*) em Itirapina, São Paulo, e determinar a sua relação com as variações climáticas; (2) estudar o padrão de formação dos anéis de crescimento, procurando encontrar sinais que possibilitem o entendimento da influência do clima sobre a fenologia e o crescimento de ambas as espécies; (3) verificar a influência do clima sobre a fenologia e o crescimento das espécies através do padrão de isótopos estáveis de carbono e oxigênio. No capítulo 1 verificamos que ambas as espécies tem o crescimento influenciado pelo clima e sazonalidade do Cerrado, respondendo a diferentes variáveis climáticas. No capítulo 2 analisamos o padrão de isótopos estáveis de carbono e oxigênio ao longo dos últimos oito anos e sua relação com a fenologia vegetativa de *Pterodon pubescens*, mostrando a influência da sazonalidade climática a nível fenológico, anatômico e fisiológico. A espécie estudada se mostrou uma muito promissora para o estudo das mudanças climáticas e seus efeitos na ecologia vegetal do Cerrado.

Palavras chave: Fenologia, Dendrocronologia, Isótopos estáveis, Cerrado, Mudanças climáticas.

Abstract

Phenology studies the occurrence of repetitive biological events and their relationship especially with climate, and also with biotic factors such as pollinators and seed dispersers. Dendrochronology studies the growth rings of trees, and the response of trees to climate change, allowing also estimating the age of the tree and revealing information about its ecology. Furthermore, dendrochronology allows the reconstruction of the climate through the analysis of the anatomy of the stem, since plants record, in the form of growth rings, signals of temperature changes and precipitation that affect their growth and dormancy. Within the growth rings it is possible to find the pattern of carbon and oxygen isotopes, which can reveal important ecological information such as the influence of seasonality on plant growth and phenology, as well as the source of water use, seasonality and climate changes. In this sense, the cyclic rings and isotopes of carbon and oxygen are anatomical and physiological responses that, related to a particular reproductive or vegetative phenology activity, may allow phenological reconstruction and the evaluation of climatic factors influencing these reconstructed patterns, allowing the understanding of climate changes effects on plants. This study aimed to (1) analyze eight years of phenology of Fabaceae (*Anadenanthera peregina* var *falcata* and *Pterodon pubescens*) in Itirapina, São Paulo, and determine its relationship to climate variations; (2) study the pattern formation of growth rings, looking for signs that enable the understanding of the influence of climate on the phenology and growth of both species; (3) determine the influence of climate on the phenology and growth of the species through the pattern of carbon and oxygen stable isotopes. In Chapter 1 we found that both species have their growth influenced by climate and seasonality of the Cerrado, responding to different climate variables. In Chapter 2 we analyzed the pattern of stable isotopes of carbon and oxygen over the past eight years and its relation to vegetative phenology of *Pterodon pubescens*, showing the influence of climatic seasonality on phenological, anatomical and physiological level. The studied species showed to be very promising for the study of climate change and its effects on plant ecology.

Key words: Phenology, Dendrochronology, Stable isotopes, Cerrado, Climate changes.

Climate influence on phenology, growth pattern and stable isotopes of two Fabaceae species in the Brazilian cerrado

General Introduction

The year of 2007 was very important not only for conservation bioscientists, but also for the population in general, once the Fourth Report from the International Panel of Climate Change was published affirming that the climate changes are caused by anthropogenic activities, that release the main greenhouse gas: the carbon dioxide (CO_2) (IPCC 2007). The CO_2 concentrations increased from 280 ppm before the industrial revolution to 379 ppm in 2005, reaching a higher concentration than the natural ones in the last 650 thousand years (IPCC 2007).

Changes of the soil use and the use of fossil fuels are among the mainly anthropogenic sources of CO2 (IPCC 2007). The deforestation releases around 1.6 billion tonnes of CO_2, which is a high quantity, even when compared to the quantity released by the use of fossil fuels (6 billion tones, Goldemberg & Lucon 2008). Besides the emissions of CO_2 as a result of deforestation contribute to climate changes, there is the fact that those forests are extremely important to the regulation of global climate (Fearnside 2001). Brazil holds 28.3% of primary reminiscent forests in the world and one of the biggest loss of forests, losing 3.1 million ha per year (FAO 2009).

One of the most deforested and threatened vegetations in Brazil is the Cerrado, the second largest Brazilian vegetation type, with 200 milion of ha, an area equivalent to that of the Western Europe (Furley 1999, Silva *et al.* 2006). The Cerrado plays an important role in the regulation of energy and mass exchange with the atmosphere (Batle-Bayer *et al.* 2010). It has suffered intense fragmentation since 1960 due to the beginning of occupation to the construction of Brasília (the federal capital), agriculture frontier expansion, livestock and construction of roads (Oliveira & Gibbs 2000, Capobianco 2002, Bach & Kelly 2004, Klink & Machado 2005, Silva *et al.* 2006). Nowadays, less than 20% of its original area if conserved (Figure 1) and less than 4% is under legal protection such as parks and reserves (Capobianco 2002, Silva et al. 2006). In the state of São Paulo, the Cerrado has suffered intense fragmentation due to the intense agricultural production, mainly sugar cane and citrus plantations, and the establishment of pastures (Durigan *et al.* 2007). Lately, it covers less than 7% of its original area in the state, being only found as isolated fragments. Amongst those fragments, less than 10% is under conservationist protection (Cavassan 2002). Simulations modeled the conversion of natural Cerrado into planted pastures and showed that precipitation might be reduced to 10% and medium air temperature may increase 0.5ºC in the upcoming decades (Hoffmann & Jackson 2000). The seasonality in temperature and precipitation along the year is one of the most striking characteristics across its range of distribution, defined as two climatic seasons: a warm and wet and another one cold and dry (Coutinho 2002). These changes resulted from deforestation my produce climatic changes which may influence the seasonality and the growth and phenology of the tree species found in this vegetation.

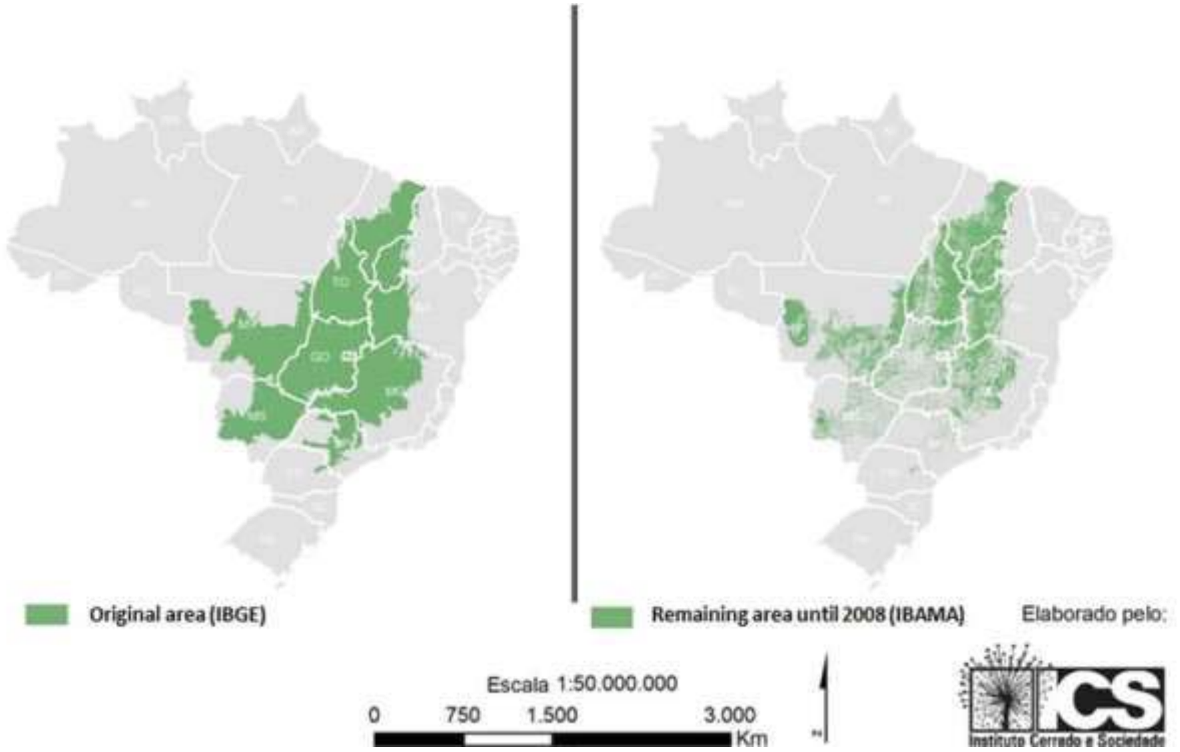

Figure 1 – Original and remaining area of Cerrado. Source: Instituo Cerrado e Sociedade.

Cerrado phenology – seasonality and climate correlates

Phenology is the area of Ecology which studies the occurrence of repetitive biological events, its relation to biotic and abiotic selective forces, as well as the interrelationship between the characterized phenophases observed and analyzed one or several species (Lieth 1974). Phenology is considered a very reliable tool to understand climate changes effects on plants (Menzel *et al.* 2006). Although many studies show the phenological pattern of Cerrado trees, the potential effect of climate changes resulted from deforestation on Cerrado phenology is still unknown. Observations in temperate vegetation have shown that phenology responds to changes in climate, especially temperature, shifting the onset of first flowering and leafing events with an early spring and in some cases a delayed leaf fall in autumn (Menzel *et al*. 2006, Schwartz *et al.* 2006, Wolkovich *et al*. 2012). For temperate forest, Wolkovich *et al.* (2012) have found an advanced leafing phenology. Price & Waser (1998) studying the response of 10 species from a subalpine meadow for 4 years under a warming experiment made by overhead heaters, also found that 6 plant species flowered early in the season responding to the one-week earlier snowmelt.

However, the climate variables that influence the phenology of temperate deciduous vegetation are different from the tropical forests (Jolly & Running, 2004). While several studies have shown that in temperate ecosystems, leaf flush and first flower are influenced by favorable temperatures and leaf senescence is determined by photoperiod (White et al. 1997; Menzel et al. 2006), in tropical forests moisture is the main trigger for flowering, leaf flush and leaf fall (Monasterio & Sarmiento, 1976, Nilsen & Muller 1981, Bullock & Solis-Magallanes 1990, Borchert, 1994, Seghieri *et al.* 1995, Morellato *et al*. 1989, Morellato *et al*. 2000, Staggemeier *et al*. 2010, Camargo *et al.* 2011).

The rainfall seasonality in the Cerrado may be considered as the trigger factor to the phenological dynamics (Felfili *et al*. 1992, Batalha 1997, Morellato *et al*. 2000, Camargo *et al*. 2011). The seasonal variation of each phenophase is considered as an adaptive strategy of the species found in this vegetation type (Oliveira & Sazima 1990, Coutinho 2002). The phenology studies of the cerrado species generally address to the community or population (Batalha 1997, Lenza & Klink 2006, Reys 2008, Camargo 2008) and less frequently the phenology of their species (Camargo *et al.* 2011, Athayde & Morellato 2013), and rarely include more than two years of observation (Camargo *et al.* 2011). Studies about phenology of cerrado vegetation confirm that climate seasonality influence both reproductive and vegetative events (Batalha 1997, Bulhão & Figueiredo, 2002, Kanegae *et al.* 2000, Lenza & Klink 2006, Gottsberger & Silberbauer-Gottsberger 2006). The leaf renewal is generally associated with the dry season not only for evergreen but also for deciduous, brevideciduous and also evergreen species (Batalha 1997, Batalha & Mantovani 2000). For instance, Damascos *et al.* (2005), observing the phenology of 3 species in the Cerrado during 3 years, found that the leaf flush is synchronous and occurs in the transition dry-to-wet season, and Borchert (1999) studied the association of trees growth and phenology, and found that both are related to the beginning of rainy season in the cerrado. Marcati *et al.*, 2000 studied the cambium activity and phenology of *Copaifera langsdorfii* and found the same pattern: while leaves are flushing, during the rainy season, the cambium is producing tree rings and while the tree is losing its leaves, the cambium is in the lowest activity, correlated with the dry season. Therefore, some studies showed a possible relation between trees vegetative phenology, cambium activity and growth rings production.

Dendrochronology and stable isotopes

One of the best available tools to study and reconstruction climate is dendrochronology. Dendrochronology is the science which studies trees response to changes in temperature and humidity, determines the age of the individuals and obtains information about tree species ecology and forest dynamics based on the analyses of tree rings and their widths patterns (Huges 2002, Worbes 2002, Alvarado 2009, Locosseli 2010, Soliz-Gamboa *et al.* 2010). The climate reconstruction is based on wood anatomy, since plant records, in the form of tree rings, signals of temperature and precipitation shifts that affected their periods of growth and dormancy (Fonti & García-Gonzales 2004, Moser *et al.* 2009, Alvarado 2009, Locosseli 2010, Soliz-Gamboa *et al.* 2010, Rozendaal & Zuidema 2010). Therefore, dendrochronology may play an essential role to understand how woody species in tropical regions are responding to historical climate changes, and its consequences for plant biology, ecology, and climatology (Wehr & Tomazello-Filho 2000, Mattos *et al.* 2004, Marcati *et al.* 2006, Locosseli 2010).

The study of growth rings has particular advantages such as to be registered annually, provide continuous record easily dated by counting rings (Managave *et al.* 2010), may encompass a wide geographic area of plant distribution. The seasonality in the growth rate of trees driven by the variability of climatic factors can result in well-defined growth rings (Managave *et al.* 2010). Rings of individual trees are reliable records of the signatures from current and past climate and consequently provide an opportunity to decipher the variation of climatic parameters for a period equivalent to the lifetime of the tree (Managave *et al.* 2010). Therefore, growth rings provide great insights on the mechanisms of how climate changes influence plants (Rozendaal & Zuidema 2010).

Growth rings and their associated vessel areas have a strong association with precipitation and temperature (Hughes 2002, Fonti *et al.* 2007, Campelo *et al.* 2010). Which are affected by rising atmospheric CO_2 concentrations (Locosselli 2013). Besides the study of growth rings and vessels, the $\delta^{13}C$ (relative proportion of carbon ^{12}C and ^{13}C) allows the direct study of the effects of rising CO_2 on plants, and has obtained very promising results (Brienen *et al.* 2011). Carbon stable isotopes are widely used in dendrochronological studies through the analysis of the relationship between ^{13}C and ^{12}C (delta ^{13}C or $\delta^{13}C$) (McCarroll & Loader 2004). Plants with C_3 photosynthetic types are able to discriminate carbon isotopes through two fractionation processes (McCarroll & Loader 2004) that can change the $\delta^{13}C$, in relation to atmospheric content: (1) the first fractionation process happens in the moment of the gas diffusion through the stomatal aperture, privileging the entering of ^{12}C isotope instead ^{13}C; (2) the fractionation process that occurs during photosynthesis, in which carbon is determined by the enzyme RUBISCO, which has preference for the lighter isotope (^{12}C) (Marino & McElroy 1991). A decrease in the value of $\delta^{13}C$ from photosynthetic products (in C3 plants -20 ‰ to -30 ‰) compared to the atmospheric average (-8 ‰) results from both fractionation processes (McCarroll & Loader 2004).

Historical records of atmospheric $\delta^{13}C$ enable the conclusion that $\delta^{13}C$ valued are not stable over time, but show a tendency to decrease in the last century (Friedli *et al.* 1986). The series of $\delta^{13}C$ record the climatic influences of temperature and precipitation and their reaction towards increased atmospheric CO_2 concentrations, which may differ from those signs found in growth rings (Andreu *et al.* 2008, Ferrio *et al.* 2003, Guo & Xie 2006, Levanic *et al.* 2008).

It is also possible to calculate, from the carbon isotopes ratios, the plant intrinsic efficiency in water use (Wi). This efficiency is the ratio of fixed carbon and the amount of lost water, since both processes are regulated by the RUBISCO's and stomatal activity (McCarroll & Loader 2004). Many studies show that the efficiency of water use is following the raising atmospheric CO_2 concentration rates, increasing especially after 1950 (Bert *et al.* 1997, Feng 1999, Hieltz *et al.* 2005, Silva *et al.* 2009). Also, the intrinsic efficiency of water use has increased in recent decades due to the increase in atmospheric CO_2 concentration (Feng 1999, Hieltz *et al.* 2005, Silva *et al.* 2009) and it may be reflected in tree growth rings.

Considering the importance of phenology and dendrochronology to detect climate change effects on plants, and the need to understand its effects on cerrado, the general aim of this research was to study the phenology, growth rings and carbon and oxygen stable isotope ratios and discuss the potential indicators of climate change effects of *Anadenanthera peregrina var falcata* and *Pterodon pubescens*.

The specific aims are:

- To determine how climate variables may influence the phenological pattern of cerrado species
- To verify how climate can be assessed by growth rings width patterns
- To correlate the phenological activity to the growth ring widths pattern, verifying the influence of climate on stable isotopes and plant physiology

- We expect that both species will provide important information about climate effects in different levels, specially seasonality and its effect on plants growth and phenology. Once both species are known to be brevideciduous, we expect that their growth and phenology are strongly influenced by rainfall as well as temperature seasonality.

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Climate, phenology and growth rings of *Anadenanthera peregrina* var *falcata* in the Brazilian cerrado

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Introduction

Cerrado is the second largest Brazilian vegetation type and plays an important role in the regulation of energy and mass exchange with the atmosphere (Batle-bayer *et al.* 2010). It covers an area with more than 2 million km² and is one of the richest and most threatened vegetations in the world (Souza & Habermann 2012). The seasonality in temperature and precipitation along the year is one of the most striking characteristics across its range of distribution, defined as two climatic seasons: one warm and wet and another one cold and dry seasons (Coutinho 2002). Cerrado, as a consequence of its large territorial extension and flat terrains, is the main national agricultural frontier, and has suffered intense fragmentation since 1950 (Klink & Machado 2005). Simulations modeling the conversion of natural Cerrado into planted pastures show that precipitation might be reduced to 10% and medium air temperature may increase 0.5ºC in the upcoming decades (Hoffmann & Jackson 2000). These changes in climate may provoke shifts in trees growth and phenology, both known to be strongly influenced by temperature in temperate regions (Menzel *et al.* 2006, Worbes 1999, D'arrigo *et al.* 2006, Esper *et al.* 2002, Briffa 2000) and precipitation in tropical regions (Borchert 1999, Morellato 2000, Marcati 2000, Brienen & Zuidema 2005, Schöngart *et al.* 2006, Campelo *et al.* 2010).

Phenology studies the occurrence of repetitive biological events, its relation to biotic and abiotic selective forces, as well as the interrelationship between the phenophases observed in one or several species (Lieth 1974). The phenology of cerrado species indicate that plants present seasonality for both reproductive and vegetative phenophases (Batalha 1997, Bulhão & Figueiredo 2002, Kanegae *et al.* 2000, Lenza & Klink 2006, Gottsberger & Silberbauer-Gottsberger 2006). In Cerrado, the leaf renewal is generally associated with the dry season (Batalha 1997, Batalha & Mantovani 2000, Lenza & Klink 2006). Flowering and fruiting activity in wind dispersed species occur in the dry or dry-to-wet season transition and has been related to rainfall and temperature (Borchert 1999, Gottsberger & Silberbauer-Gottsberger 2006, Lenza & Klink 2006). The leaf exchange in the cerrado occurs mainly in the dry season and day-length and precipitation are suggested as the main drivers of leafing phenology (Longman & Jenik 1974, Leigh & Windsor 1982, Morellato *et al.* 1989, Borchert *et al.* 2002).

Blooming and leaf change may be also related to the formation and growth of rings in tree wood (Borchert 1999, Marcati 2000, Schöngart *et al.* 2002). Dendrochronlogy is the study of the chronological sequence of growth rings originated from seasonality (Worbes 2002) that allows the reconstruction of reproductive and vegetative dynamic on plants based on wood rings, generating series extracted from the rings patterns which may be related to past climate (Worbes 2002) and may allow the phenological reconstruction based on rings formation. Growth rings have a strong relation with precipitation and temperature (Hughes 2002, Fonti *et al.* 2007, Campelo *et al.* 2010), climatic variables which are affected by rising atmospheric CO_2 concentrations (IPCC 2007).

The seasonality influences trees growth once the cambium activity is known to be very low or almost absent when there is a dry season (precipitation below 50 mm) of at least two months (Worbes 1999), allowing the formation of growth rings (Brienen & Zuidema 2005). Therefore, dendrochronology is, together with phenology, one of the best tools to understand tree responses to changes in climate, besides, determine the age of the individuals and obtain information about tree species ecology and forest dynamics (Huges 2002, Worbes 2002, Alvarado 2009, Soliz-Gamboa *et al.* 2010). Dendrochronological studies in tropical forests have found strong correlations between growth rings width and precipitation and soil moisture (Reich & Borchert 1984, Enquist & Leffler 2001, Brienen & Zuidema 2005). Furthermore, several studies have also shown that tropical forests are sensitive to drought and changes in precipitation (Frankie *et al.* 1974, Opler *et al.* 1976, Stowe & Brown 1981, Condit *et al.* 1995, Swaine 1996, Condit 1998, Nepstad *et al.* 1999, Engelbrecht *et al.* 2007, Phillips *et al.* 2009, Brienen & Zuidema 2005, Brienen *et al.* 2012). Brienen & Zuidema (2005) analyzed 154 trees belonging to six rain forest species in the Bolivian Amazon and found a relationship between tree growth and rainfall in certain periods of the year: while some species showed sensitiveness to the rainfall at the end of the previous growing season, other species showed a strong relationship with rainfall at the beginning of the rainy season. The relationship between growth rings widths and rainfall is also confirmed by Shongart *et al.* (2006), Brienen *et al.* (2010), Soliz-Gamboa *et al.* (2011), Locosselli *et al.* (2013).

Although studies addressing the dendrochronology of Cerrado species are rare, a few studies have found a strong correlation between growth-rings and climate variables. Toledo *et al.* (2011), comparing wood increment between forest and savanna ecotypes, found that the Cerrado ecotype presents higher wood density and smaller rings, understood as resistance against cavitation and drought. Borchert (1999) found a strong correlation between radial increment, precipitation and vegetative phenology. Marcati (2000) reports a relation between precipitation, growth-ring formation and leaf flush and leaf fall for two different species from Cerrado, and Locosselli *et al.* (2013) also found a strong correlation between growth-rings width and precipitation.

The present study aimed to understand the influence of climate on phenology and growth rings within a context of climate changes from *Anadenanthera peregrine* var *falcata,* typical and one of the most important species of Fabaceae from the Brazilian Cerrado.

Materials and Methods

Study area

This research took place in a cerrado remnant with 260 ha, located at Fazenda São José da Conquista, in the municipality of Itirapina, São Paulo State, Southeastern Brazil (22º 10' 31.41"S; 47° 52' 26.1 3"W, 610 m of altitude). The fragmente was formed about 30 years ago and it presents a rectangular shape with sides facing the four cardinal points. The vegetation is a cerrado *sensu stricto* (Coutinho 1978), a savanna-like vegetation, characterized by the presence of small, leaning, tortuous trees, with irregular and twisted ramifications (Ribeiro & Walter 1998). This vegetation holds a discontinuous canopy of woody plants reaching six to seven meters high with a continuous herbaceous layer; the cerrado becomes denser at some parts with some trees reaching up to 12 meters high and reduced herbaceous layer (Camargo *et al.* 2011, Reys *et al.* 2013).

Climate

The average for the eight years was calculated, showing the mean pattern of rainfall, temperature and solar radiation. Graphs showing the climatic pattern from 2005 to 2012 were produced with daily, decimal and monthly intervals for rainfall amount and temperature (maximum, mean and minimum).

Study species

Anadenanthera peregrina (L.) Speg. *var falcata* Benth.

The species *Anadenanthera peregrina var falcata* (Fabaceae) or angico-do-cerrado, hereafter just *Anadenanthera*, is one of the most important trees in the Brazilian cerrado due to its size, relative frequence and abundance (Batalha 1997, Batalha & Mantovani 2000, Bartimachi *et al.* 2008). This species is dominant and the most occurring species in the studied area, presenting individuals spread all over the fragment, being found in the fragment interior as well as in its edges. This species presents a very thick and rigid bark and thin canopy, flowering in September and October, the transition from dry and wet season) and the seeds are self-dispersed (Teixeira *et al.* 2002). There are few studies about this species (Bartimachi *et al.* 2008) and it is even rarer studies about its phenology (but see Athayde & Morellato 2013) and dendrochronology. This research is the first one to connect phenology and dendrochronology for this species.

Phenology and climate

Phenological observations were recorded in 36 transects of 25 x 2 m, with at least 50 m of distance between them. Transects were distributed along the interior and the edge of East and South faces in the Cerrado fragment. All individuals of *Anadenanthera* with circumference at the stem base equal or larger than 3 cm were sampled, totalizing 60 trees.

We conducted phenological observations at monthly intervals from January 2005 to December 2012 on the following phenophases: flower buds, anthesis or flowering, unripe fruiting, ripe fruits or fruiting, leaf flush and leaf fall. We estimated the intensity of each phenophase using a semi-quantitative index of intensity ranging from 0 (absence) to 2 (peak) (Opler et al. 1976, Morellato et al. 2000). A population is considered highly synchronic when more the 60% of the individuals are producing the same phenophase, few synchronic when the percentage of individuals is between 20% and 60% and asynchronic for 20% individuals or less (Benke & Morellato 2002).

The climate of the cerrado is seasonal, with a hot and wet season from October to March and a dry and less warm season from April to September (Figure 1). During all the studied period, from January 2005 to December 2012, the climate was similar among the years and confirmed the seasonal pattern described for the region (Camargo *et al.* 2011). The climatic data were provided by CHREA (Centro de Recursos Hídricos e Ecologia Aplicada), of University of São Paulo (USP), located 4 km far away from the study area.

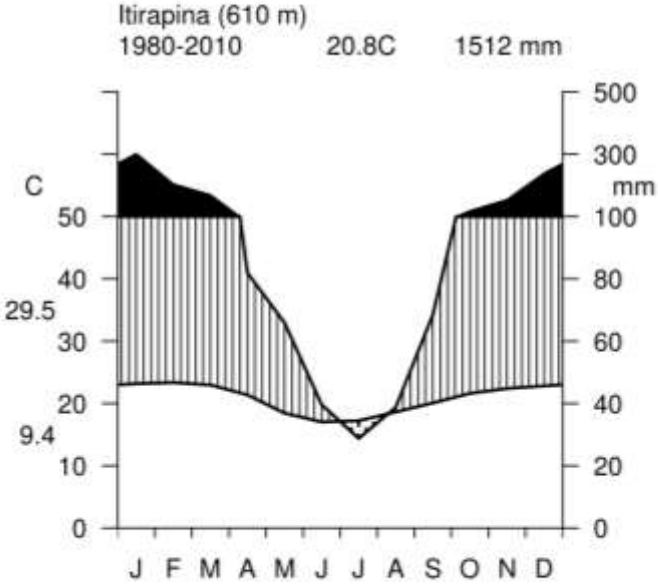


Figure 1– Climate diagram showing a 30 years average (Rainfall and Temperature) in Itirapina, São Paulo (Souza *et al.* 2015 (*in press)*).

Growth-rings

We sampled between 2 and 4 wood cores per tree with a motorized auger from the main trunks of 10 individuals of *Anadenanthera* inside the phenology transects which were selected through the trunk diameter (the largest trees were selected to be sampled). The cores were drought naturally and polished with different grids of sandpaper (from 50 to 2000) (Urquiza 2008, Alvarado 2009, Soliz-Gamboa *et al.* 2010). All the parenchyma band rings were marked and measured using LINTab (Drobyshev *et al.* 2010) and TSAPWin program, to analyze the similarities between the radios and to compare the behavior of series as well as to verify which rings could be intraspecific markers (Locosselli *et al.* 2013). After the measurement, the growth rings had their widths processed by the program COFECHA (Soliz-Gamboa *et al.* 2010, Locosseli *et al.* 2013), which indicates the similarity between the individuals.

In order to build a relation between growth rings and phenological activity, we focused on the last 8 rings that represent the whole period of phenological data. The species *Anadenanthera peregrina* var *falcata* is brevideciduous and presents well defined growth rings indicated by a parenchyma band (Marcati *et al*. 2006).

Analyses among climate, phenology and growth rings

In order to understand the relation between climate and phenological activity for both species, Spearman correlations were developed using the program Statistica, analyzing the correlation between the last 8 years of phenological and climate monthly data. Besides that, we used Pearson's correlation to understand the influence of climate on the growth rings residual chronology in the last 30 years for both species.

Results

Phenology and climate

The average precipitation for the 8 years of study presented the same patterns of the 30-year average climate, showing a wet season from October to March (precipitation above 100mm) and a dry season from April to September, with precipitation generally bellow 60 mm (figure 2A). Analysing the climatic pattern in a daily (Figure 2B) and decimal interval (Figure 2C) we can find a rainfall pattern throughout the years with some important variations such as the peak of rainfall in January 2011 (Figure 2B) and 2012 (Figure 2C).

Although the species has presented a seasonal phenological pattern for vegetative phenophases, there was not considerable reproductive activity especially during the studied period (Figure 3), with no synchrony for the monthly interval. Therefore, we decided to focus on vegetative phenology and its relation with climatic variables.

Anadenanthera presented the highest activity of leaf flush at the end of the dry season or in the transition from dry to the wet season, in September, while the leaf fall peak of activity was in the middle of dry season, in July, for the 8 years of study (Figure 4). The length of the leaf fall activity of *Anadenanthera* was 6 months (Fig. 4). An exception occurred between 2009 and 2011, when more than one peak occurred in different months for both phenophases and the pattern was not so clear (Fig. 4). The 10-day average rainfall pattern showed a longer wet season in 2009 and a peak of precipitation in 2011 (Figure 5) with a variation in leaf flush and fall activity. The activity of leaf fall and leaf flush were complementary, as soon as leaf fall finishes, the species started to flush new leaves.

Anadenanthera did not shown any correlation between leaf flush and climate variables (Table 1). The spearman correlation between phenology and climate showed that the species present a negative correlation between phenological activity of leaf fall and all climatic variables (Tmax, Tmean, Tmin and rainfall) for all the years (Table 1). However *Anadenanthera* did not present any influence of climate on leaf flush activity (Table 1).

Comparing monthly leaf flush activity from August and September (onset of flushing) with monthly climate data, specifically the most rainy months of the wet season (January, February and March) it was possible to find a significant correlation between the leaf flush activity of *Anadenanthera* in August with maximum temperature from August and rainfall amount from March (Table 2). Besides that, the leaf flush activity from September was significantly correlated with the maximum temperature from September of the previous year and a negative correlation with the rainfall amount from February (Table 2).

Figure 2A – Average rainfall (2005 – 2012) in Itirapina, São Paulo, Southeastern Brazil. Light blue spots indicate rainfall amount below 50 mm, while dark blue spots indicate rainfall amount below 100 mm.

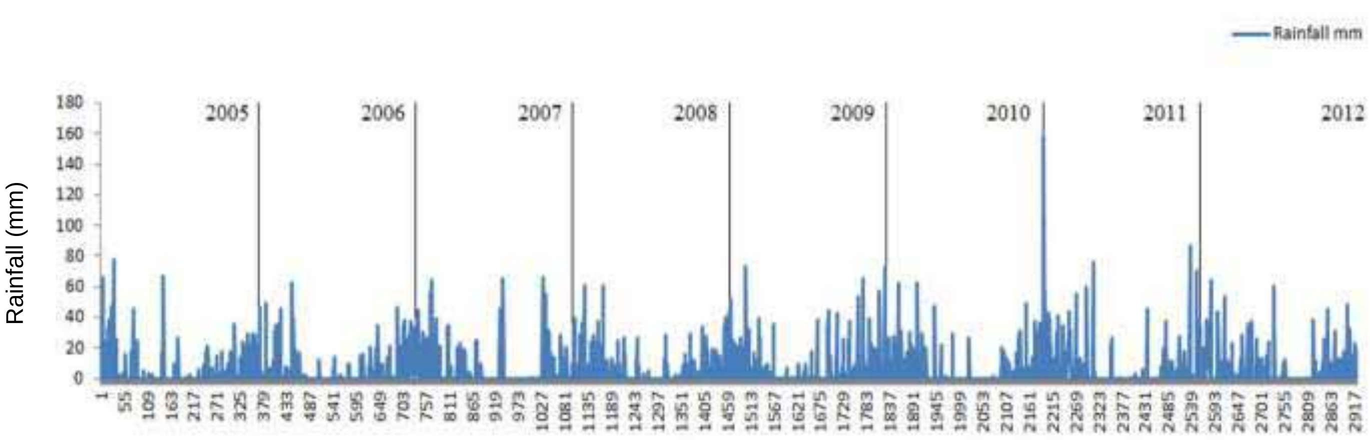


Figure 2B – Daily rainfall pattern from January 2005 until December 2012 in Itirapina, São Paulo, Southeastern Brazil.

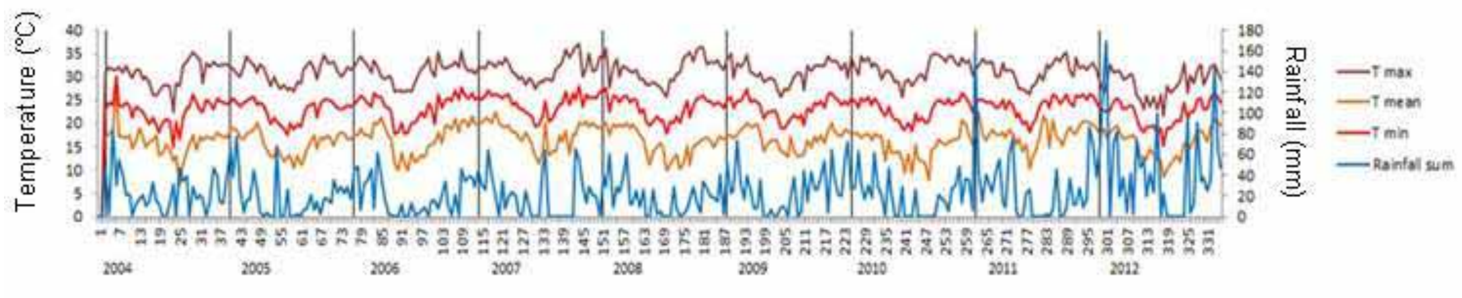


Figure 2C – Decimal rainfall (sum), maximum temperature, mean temperature and minimum temperature from January 2004 to December 2012 in Itirapina, São Paulo, Southeastern Brazil.

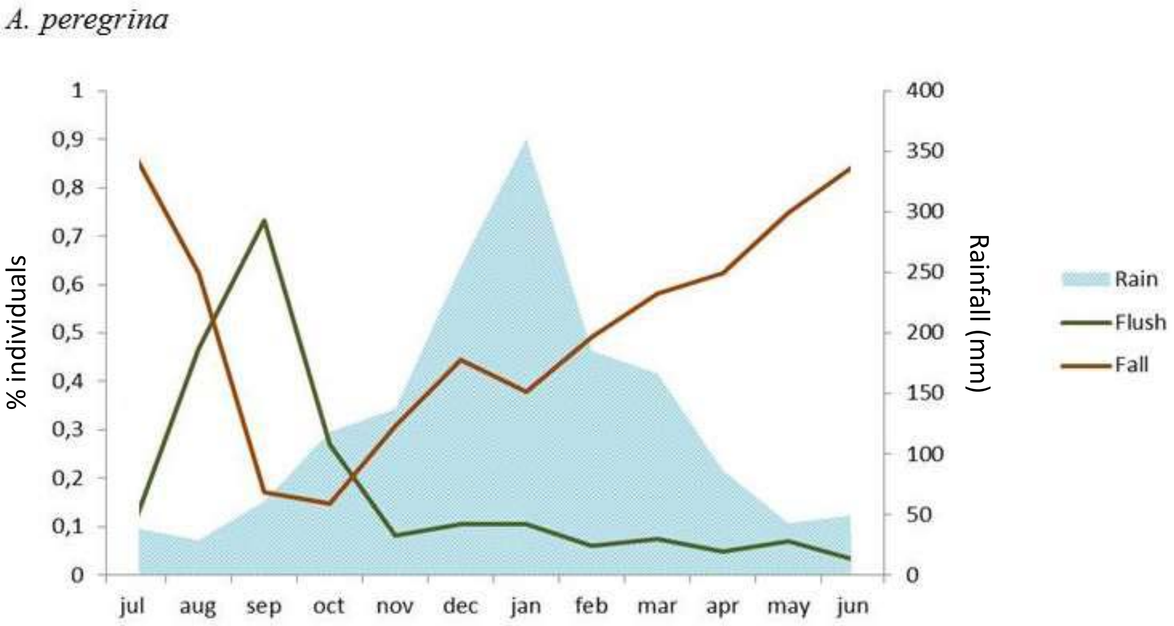


Figure 3 – Average phenological activity of leaf flush and leaf fall for *Anadenanthera* (above) and *Pterodon* (below) and rainfall pattern from January 2005 until December 2012.

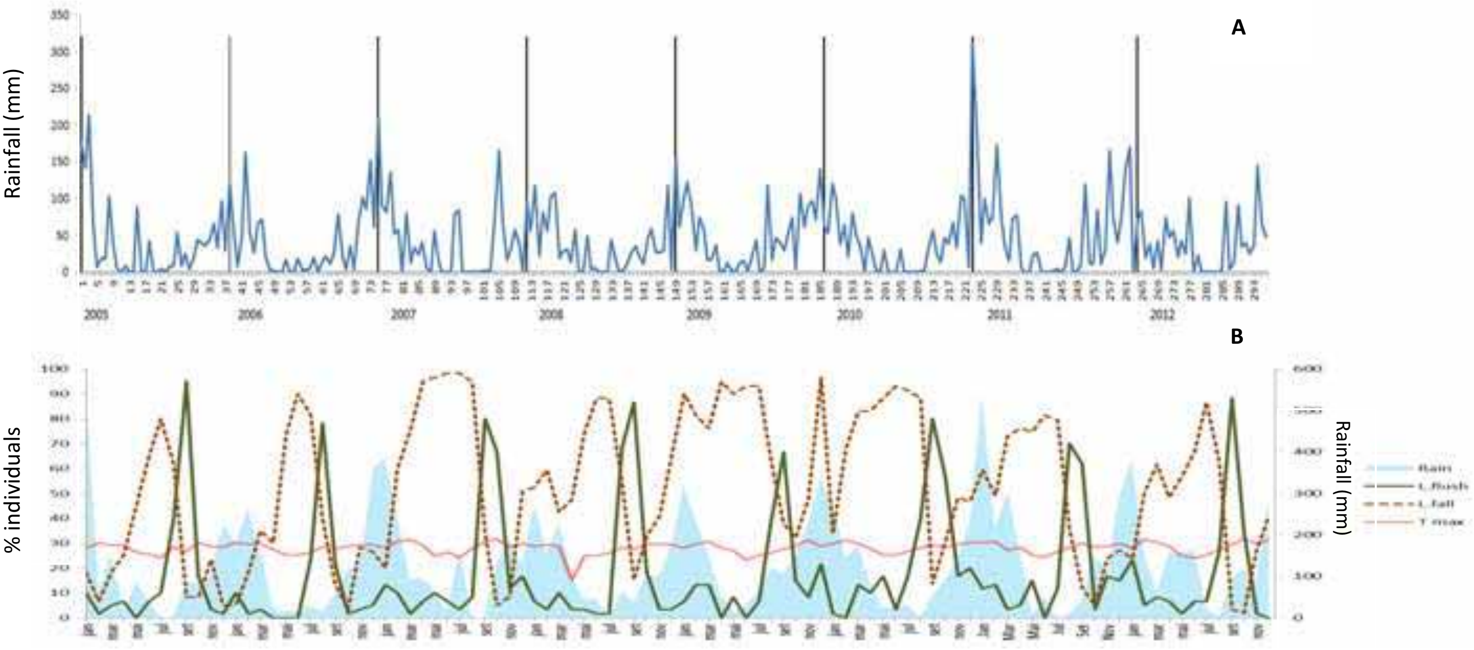
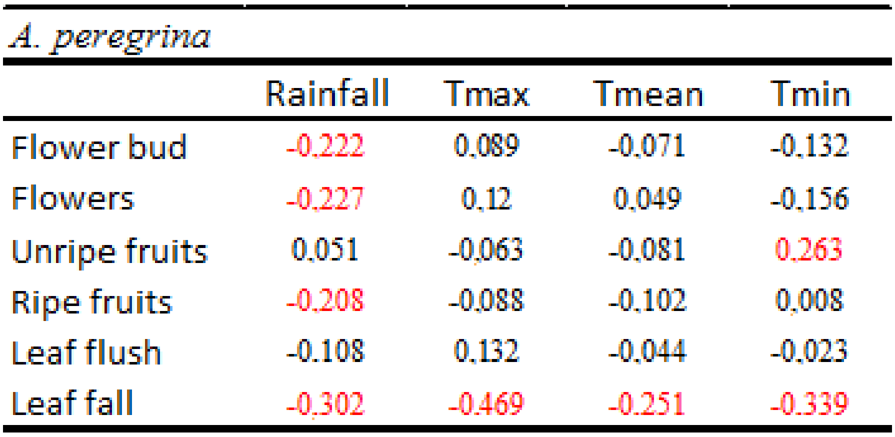


Figure 4 – Patterns of (A) decimal rainfall, leaf flush (green line) and leaf fall (dashed brown line) for (B) *Anadenanthera*, monthly rainfall and maximum temperature (blue shadow) from January 2005 until December 2012, in the cerrado, Southestern Brazil.

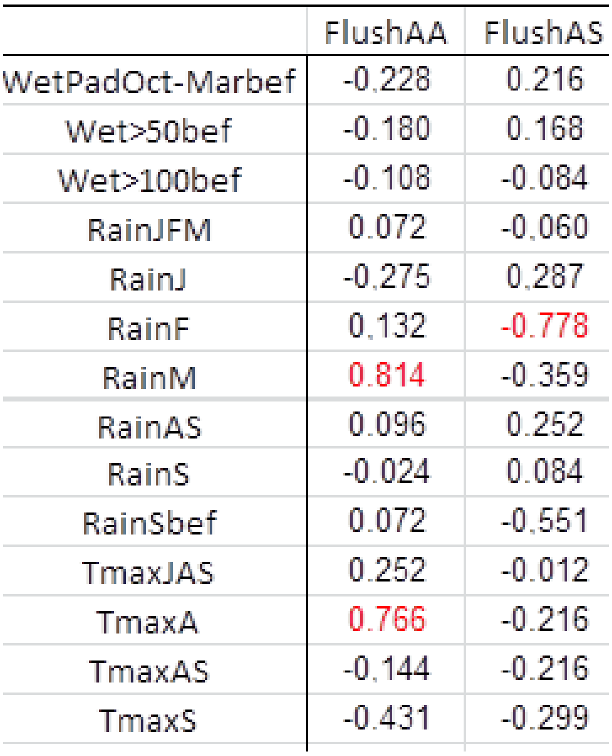
Table 1 – Spearman correlation between the phenological activity of *Anadenanthera peregrina* and *Pterodon pubescens* and climate variables (Tmax, Tmean, Tmin and rainfall) from January 2005 until December 2012 – the significant results are shown in red.



A. peregrina

	Rainfall	Tmax	Tmean	Tmin
Flower bud	-0.222	0.089	-0.071	-0.132
Flowers	-0.227	0.12	0.049	-0.156
Unripe fruits	0.051	-0.063	-0.081	0.263
Ripe fruits	-0.208	-0.088	-0.102	0.008
Leaf flush	-0.108	0.132	-0.044	-0.023
Leaf fall	-0.302	-0.469	-0.251	-0.339

Table 2 – Spearman correlation between the annual vegetative phenological activity from August (FlushAA) and September (Flush AS) for *Anadenanthera*, and the annual average of climate variables during wet season: Tmax from July (J), August (A) and September (S) and rainfall amount from January (J), February (F), March (M), August and September (AS), September (S) and September from the previous year (Sbef) from January 2005 until December 2012.



	FlushAA	FlushAS
WetPadOct-Marbef	-0.228	0.216
Wet>50bef	-0.180	0.168
Wet>100bef	-0.108	-0.084
RainJFM	0.072	-0.060
RainJ	-0.275	0.287
RainF	0.132	-0.778
RainM	0.814	-0.359
RainAS	0.096	0.252
RainS	-0.024	0.084
RainSbef	0.072	-0.551
TmaxJAS	0.252	-0.012
TmaxA	0.766	-0.216
TmaxAS	-0.144	-0.216
TmaxS	-0.431	-0.299

Growth-rings distinctiveness and structure

Anadenanthera presented different structure of rings: marginal parenchyma bands, fibre zones and variations in vessel size (semi porosity) (Fig. 6), which were confirmed through anatomical cuts (Fig. 7 and 8).

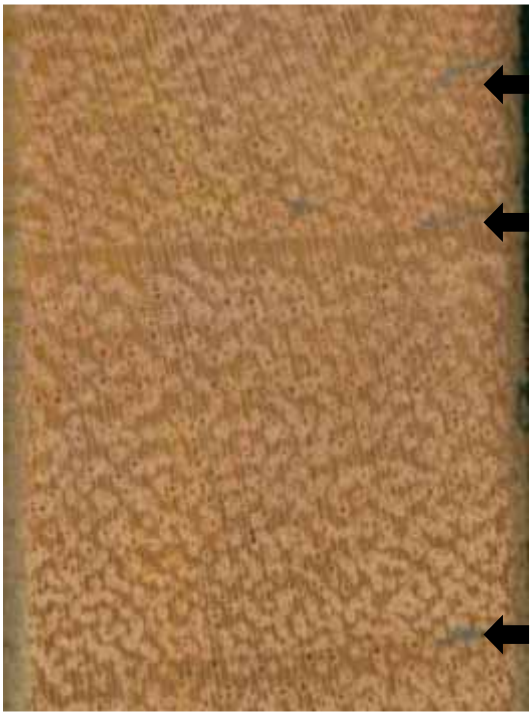


Figure 5 – Growth rings from *Anadenathera peregrina*. Arrows point the marginal parenchyma band, fibre zone and semi porosity from the rings of *Anadenathera*.

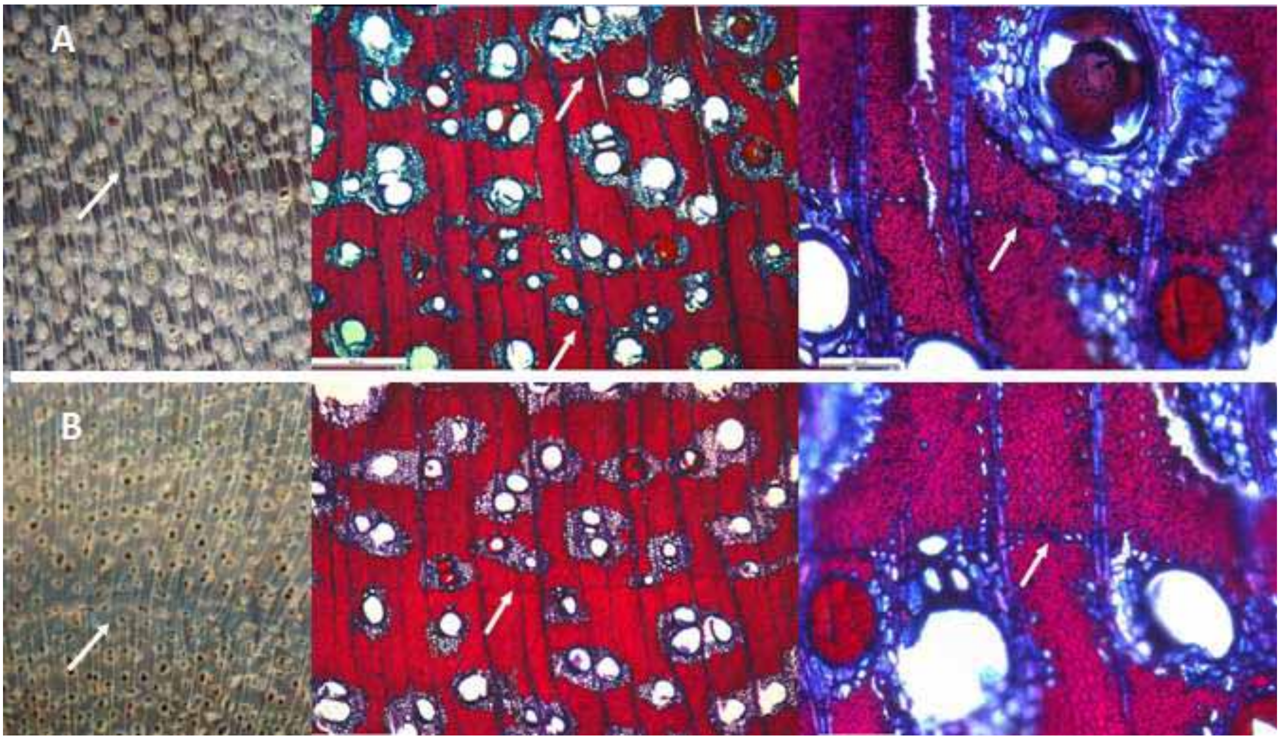


Figure 6 – Anatomical cuts of growth rings of *Anadenanthera*, showing the parenchyma band (A) and the semi porosity (B) in the wood core and in the microscope images.

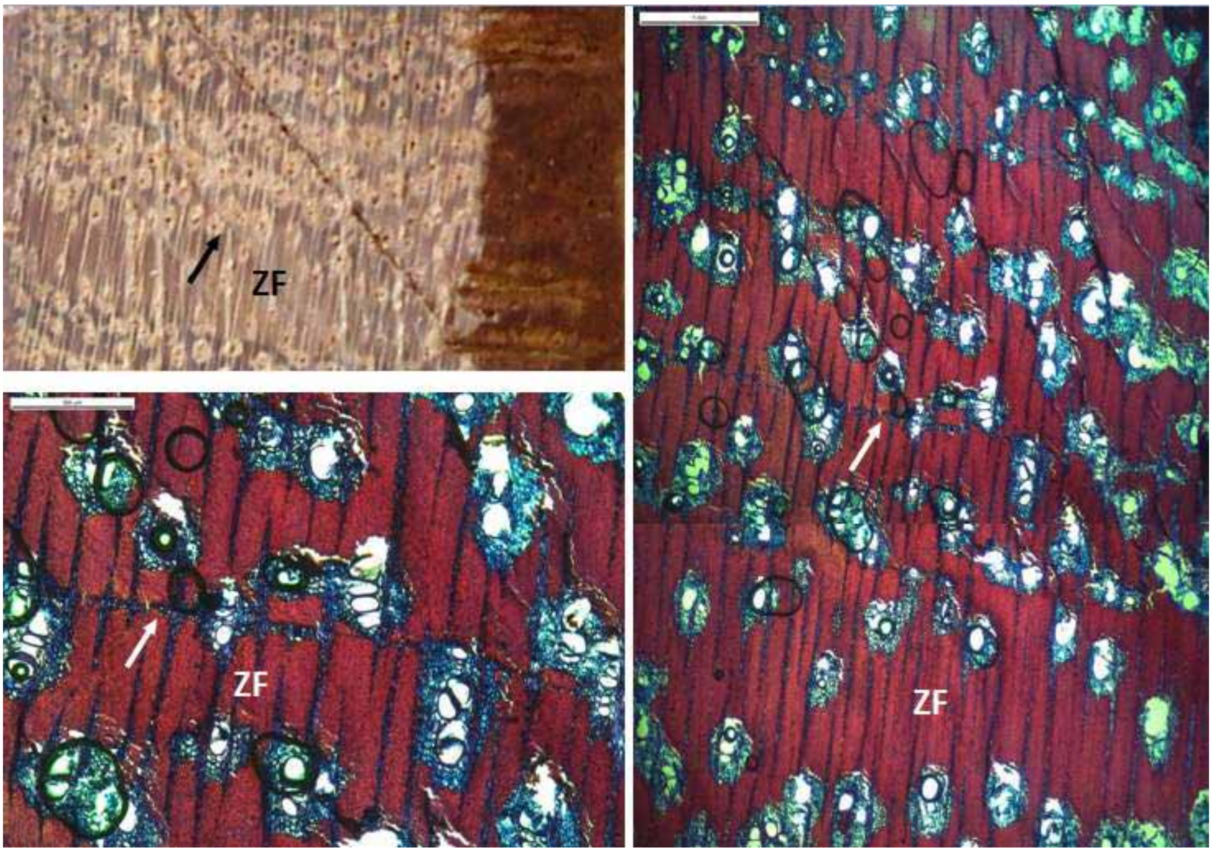


Figure 7 – Anatomical cuts of growth rings of *Anadenanthera*, showing clockwise the fibrous zone (ZF) in the wood core (black arrow), and the fibrous zone (ZF) in the anatomical cut, showing the parenchyma line (white arrow) in the microscope image.

Chronology

The residual chronologies correlations with climate showed that *Anadenathera* was influenced by the amount rainfall in September and January (negative) of the current year (Fig. 9).

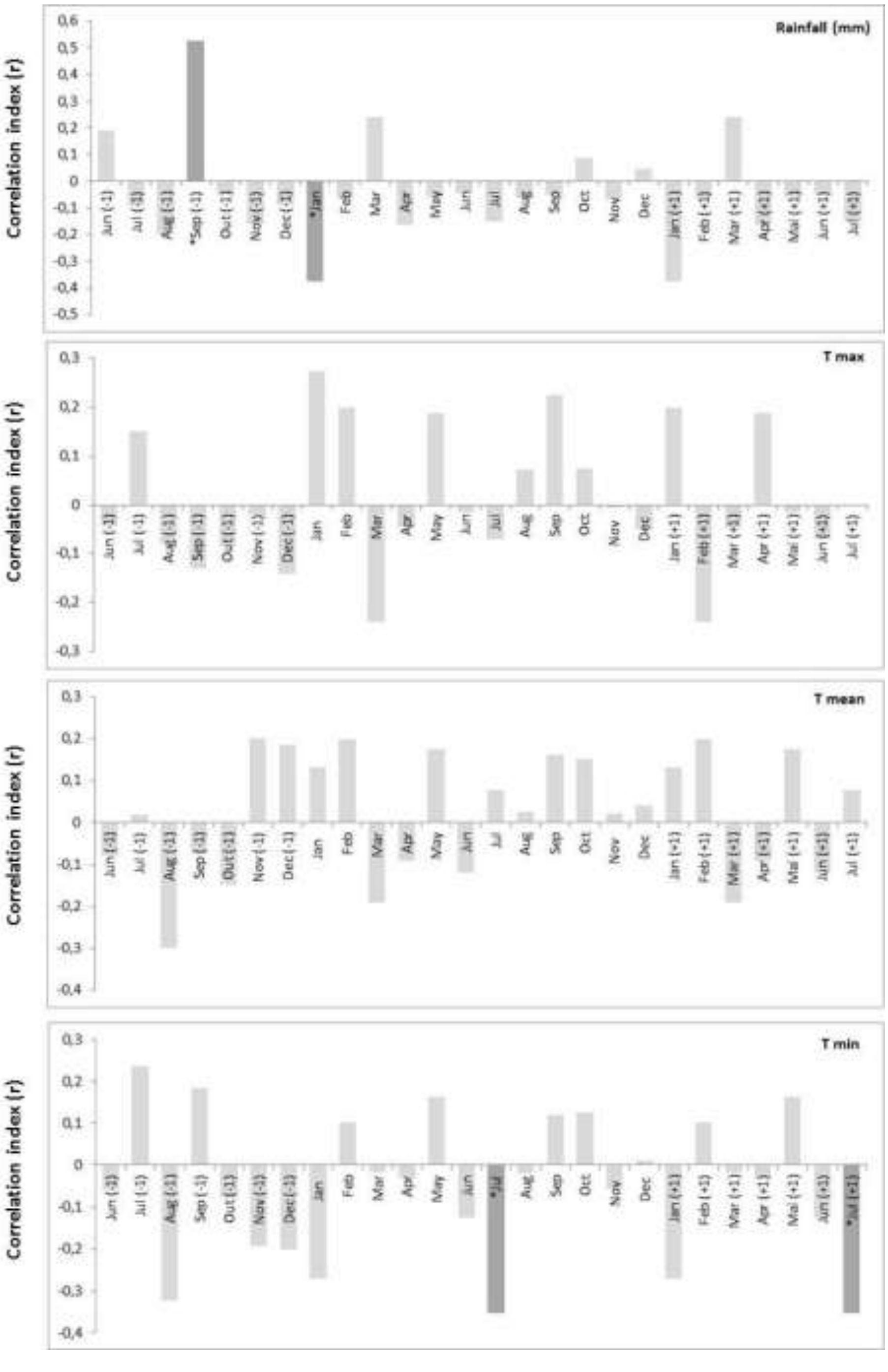


Figure 8 – Correlation between residual chronology from *Anadenanthera* and climate variables. Values marked with (*) shows significant results (<0.05).

Discussion

Anadenanthera peregrina leaf flush and leaf fall patterns during the studied period were seasonal and related to rainfall seasonality, confirming the expected phenological pattern for cerrado species. The alternation between dry and wet seasons is considered the most important factor driving the phenology of many different species of cerrado (Batalha & Mantovani 2000, Lenza & Klink 2006). The seasonality is considered an adaptive strategy of cerrado species (Oliveira 2008). The distinct leaf flushing period beginning at the end of the dry season (August and September) and finishing by November-December, prior to the start of torrential rainfall in January/February, supports the general view that Cerrado woody plants flush (and reach leaf maturity) avoiding the herbivory peak, during the warm and wet season, as well as the nutritional leaf leaching (Mantovani & Martins 1988). The leaf flush peaking in the dry season and before wet season is known to allow trees to increase carbon gain in the growth period besides achieving high photosynthetic potential (Franco *et al.* 2005). In dry tropical zones, such as the Sahelian and the Cerrado, leafing is significantly correlated with cumulative rainfall because of the marked periodicity of the rainy season (Seghieri *et al.* 2009).

On the other hand, the peak of leaf fall occurred in the dry season stimulated by drought and/or nitrogen deficiency, is also found by other researches for cerrado species (Batalha 1997, Marcati, 2000, Camargo et al. 2011, Batalha 1997, Antunes & Ribeiro 1999, Lenza & Klink 2006). Leaf fall may represent a defense mechanism against water loss, avoiding the death of the individual in stressful times as the dry season (Batalha 1997, Antunes & Ribeiro 1999, Lenza & Klink 2006, Camargo *et al*. 2011).

While in temperate ecosystems leaf flush has been shown to occur in response to favorable temperatures and leaf senescence determined by photoperiod (White *et al.* 1997), several studies have pointed that in tropical regions both leafing and senescence are primarily controlled by moisture (Bullock & Solis-Magallanes 1990, Borchert 1994, Seghieri *et al*. 1995). Species adapted to dry regions have rapid leaf flushes at the onset of the rainy season as an adaptation to long dry periods (Monasterio & Sarmiento 1976), while they shed their leaves as long as available soil water decreases in respond to the lack of precipitation (Nilsen & Muller 1981, Childes 1989), pattern found for both species. Leaf flush rapidly starts together with the rainy season in response to the first large precipitation event (Childes 1989), whereas leaf senescence is known to respond more slowly to the decrease in water availability (Childes 1989, Borchert 1994). These adaptations optimize photosynthesis once dropping some leaves, but not all of them, allows the plant to keep on photosynthesizing during the dry season, while lowering the overall transpiration loss (De Bie *et al.* 2002).

Leaf fall is known to be correlated with the dry season, when cambium activity is extremely low or even absent, while leaf flush is supposed to be correlated with the rainfall of wet season, when cambium activity is high or increasing (Marcati 2000). For Cerrado species, it is also suggested that trees can be influenced not only by rainfall amount, but also by the amount of water storage in the soil, possibly showing a different relation to climatic variables. Once the wood production is associated with cambial activity which is influenced by precipitation, it is possible to analyze the influence of rainfall amount in growth rings sizes and, consequently, in phenological activity.

Our study found a strong relation between growth rings and precipitation for *Anadenanthera*, possibly correlated with reproductive phenophases or leaf fall, but not with leaf flush. The rare studies about dendrochronology of Cerrado species have found a strong correlation between growth-rings and precipitation: Toledo *et al.* 2011, who compared wood increment between forest and savanna ecotypes, found that the Cerrado ecotype presents higher wood density and smaller rings, understood as resistance against cavitation and drought; Borchert (1999) found a strong correlation between radial increment, precipitation and vegetative phenology; Marcati (2000) found a strong relation between precipitation, growth-ring formation and leaf flush and fall for two different species from Cerrado; Brienen & Zuidema (2005) analyzed the climate influence on growth rings of six different species, and found a positive relationship between growth rings and rainfall in different periods of the year; and Locosselli *et al.* (2013) also found a strong correlation between growth-rings width, precipitation and temperature.

Anadenanthera growth was influenced by the rainfall amount of the previous year, suggesting the influence of water storage in the soil, however, this species also presented a relationship between leaf flush, rainfall and maximum temperature of the actual year. Although *Anadenanthera* did not present a clear relationship between climate influence on growth and leaf flush, this species presented a clear relationship between growth rings, rainfall and temperature and leaf fall. Both species presented a strong negative correlation between growth rings pattern and minimum temperature of July (also June for *Pterodon*), period of the year when leaf fall activity reaches the maximum. The negative influence of temperature on both species chronologies shows how sensitive they are towards the hydrological stress originated from the evapotranspiration.

Based on the literature data and our results from response functions of the species, we may expect a decrease of wood production in higher temperatures, due to hydrological stress caused by temperature increase (Locosselli *et al.* 2013). The species may suffer a negative influence on their growth with the decrease of precipitation in June for *Anadenanthera*. Once CO_2 increase in atmosphere is leading to higher temperatures and drier climate (Huges 2002, Malhi *et al.* 2008, Marengo *et al.* 2008), we may expect significant effects on the growth and phenological activity of both species.

Conclusion

Anadenanthera peregrina var falcata suggested an important climate influence over its growth and phenological activity. One difficulty found in this association is the time interval of each time series. While phenology is monthly observed, growth rings data is annual, meaning that the most important data for this correlation is when the growth rings start to be produced and when the cambium decreases or stops its activity.

The answers found for the species showed that it suffers influence of rainfall; therefore, for being sensitive to climate, we may suppose that the species growth and phenology might suffer influences of climate changes over the next years.

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Growth rings, phenology and climate influence inferred by high-resolution stable isotopes: the case of *Pterodon pubescens*

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Abstract

Dendrochronology studies tree growth-rings and how trees have reacted to climate variables over time. It also estimates tree age and reveals information about its ecology. Carbon stable isotopes from growth rings are widely used in dendrochronological studies through the analysis of the relationship between ^{13}C and ^{12}C (delta ^{13}C or $\delta^{13}C$), and have strong relationship to the photosynthetic C3 type in plants. Plants are able to discriminate carbon isotopes through two fractionation processes that can change the $\delta^{13}C$, in relation to atmospheric content, and may also be related to trees phenology. Phenology studies the occurrence of repetitive biological events, its relation to climate and also biotic factors. Thus, the cyclic tree-rings, which are anatomical responses, could be related to a determined phenophases, allowing a phenological reconstruction and an evaluation of climatic factors influencing the reconstructed history, and to understand climate change effects on phenological patterns. There are still few observable evidences of increasing atmospheric CO_2 in growth rings. The analysis of stable carbon isotopes is an approach that has produced important results for the comprehension of how atmospheric CO_2 is influencing plants. The present study aimed to understand the relationship between tree phenology, seasonal patterns of stable carbon isotopes and climate in *Pterodon pubescens,* a typical species of Cerrado from Southeastern Brazil. We reconstructed a high -resolution stable carbon isotope series over the last eight years based on wood samples of three different individuals. We then linked the seasonal patterns of $\delta^{13}C$ and $\delta^{18}O$ to climate and phenophases activity. The results showed a seasonal pattern of both $\delta^{13}C$ and $\delta^{18}O$, suggesting a clear relation to leaf fall and leaf flush, both strongly linked to dry season.

KEY WORDS: Brazil; Cerrado; Climate; Stable isotopes; Growth rings; Phenology; Fabaceae.

Introduction

During leaf development, plant species have different physiological needs. For example, the young leaves use the carbon storage to satisfy energy needs for growth and leaf development, including carbon matter from other parts of the plant (Kerbauy 2004). Moreover, mature leaves store the excess carbohydrates, which in many cases are used for producing new leaves (Buckeridge *et al*. 2008). Most of the carbon fixed during photosynthesis is used in the formation of new carbohydrates which provide energy for respiration (Kerbauy 2004). When the excess of carbohydrates is not consumed in respiration activity, the plant tends to have a positive balance of CO_2 that can be applied in growth, reserve and increase of dry matter (Larcher 2000).

In the majority of plants, chloroplasts receive carbon CO_2 captured from the atmosphere and biochemically linked with the existing carbon compounds in the leaves. They form long carbon chains molecules so that when glucose and fructose are linked together, they form sucrose. On the other hand, glucose can be linked to form starch (Buckeridge 2009), which plays exclusively reserve function (Buckeridge 2000). In seasonal environments, plant species have developed strategies for the use of water and light, minimizing the effects in the carbon balance of plants (Franco *et al.* 2002). These effects, such as stomatal closure and low water use efficiency, and their relationships with climate can be extremely reliably studied through both carbon isotopes in leaves and in tree growth rings (McCarroll & Loader 2004, Helle & Schleser 2004, Brienen *et al.* 2012).

Carbon stable isotopes are widely used in dendrochronological studies through the analysis of the relationship between ^{13}C and ^{12}C (delta ^{13}C or $\delta^{13}C$), and have strong relationship to the photosynthetic C3 type in plants (McCarroll & Loader 2004). Plants are able to discriminate carbon isotopes through two fractionation processes (McCarroll &

Loader 2004) that can change the $\delta^{13}C$, in relation to atmospheric content: (1) the first fractionation process happens in the moment of the gas diffusion through the stomatal aperture, privileging the entering of ^{12}C isotope instead ^{13}C; (2) the fractionation process that occurs during photosynthesis, in which carbon is determined by the enzyme RUBISCO, which has preference for the lighter isotope (^{12}C) (Marino & McElroy 1991). A decrease in the value of $\delta^{13}C$ from photosynthetic products (in C3 plants -20 ‰ to -30 ‰) compared to the atmospheric average (-8 ‰) results from both fractionation processes (McCarroll & Loader 2004).

Carbon isotopes ratios in plant material are used to calculate the intrinsic plant water use efficiency. This efficiency is the ratio of fixed carbon per the amount of water lost, which is basically controlled by the ratio between assimilation and stomatal conductivity (McCarroll & Loader 2004). Many studies show that the efficiency of water use is following the raising atmospheric CO_2 concentration rates, increasing especially after 1950 (Bert *et al.* 1997, Feng 1999, Hietz *et al.* 2005, Silva *et al.* 2009).

Besides carbon isotopes, oxygen isotopes allow the understanding of the source water signal in the wood through the relation between ^{16}O and ^{18}O (McCarroll & Loader 2004). Analyses of these isotopes provide valuable environmental information about patterns of plant water use and climatic variation, such as seasonality in rainfall (Dawson 1993). Variation in the relationship between ^{16}O and ^{18}O ($\delta^{18}O$) shows the amount of rainfall through the source water information once the lighter isotopes in water vapor escape from liquid surfaces easier than the isotopically heavy water molecules (Roden *et al.* 2000), suggesting the amount of rainfall, temperature variation and air humidity throughout each growth ring (McCarroll & Loader 2004, Brienen *et al.* 2011, Brienen *et al*. 2012). Isotope series may corroborate Craig-Gordon and Roden models (Barbour 1997) that relate enrichment of leaf water above source water to the fractionation through stomata and leaf boundary layer, the proportional depression of water vapor pressure by the heavier $H_2^{18}O$ molecule, the oxygen isotope composition of water vapor relative to source water and scaled by the ratio of ambient to intercellular water vapor mol fraction (Barbour 2007). Therefore, important climatic information that is provided by both carbon and oxygen isotopes may be reflected in tree growth rings and phenological patterns, allowing a better understanding of climate changes effects on plants.

The formation and growth rings in wood is related to the trees phenology, or the recurrent occurrence of cyclic events in plants, such as leaf fall and flush, and the seasonality of the climate (Borchert 1999, Morellato *et al*. 2000, Schöngart *et al.* 2002). Dendrochronology studies reconstruction of climate and reproductive and vegetative phenology changes over time based on tree growth rings (Worbes *et al.* 2013), which allows to study how climate may affect phenology. Phenology is considered the easiest way to monitor and understand climate changes effects on plants (Menzel *et al.* 2006). The studies on temperate vegetation have found an earlier onset of flowering and leafing associated to increasing temperatures in the last century (Menzel *at al*. 2006, Wolkovich *et al.* 2012).

Studies about phenology of cerrado, a neotropical highly diverse vegetation, indicate that the plants present seasonality for both reproductive and vegetative phenophases presenting annual peaks of flowering, leaf fall and leaf flushing (Batalha 1997, Lenza & Klink 2006, Gottsberger & Silberbauer-gottsberger 2006). In the Cerrado, leaf renewal generally occurs during the dry season (Batalha & Mantovani 2000, Lenza & Klink 2006, Rossatto *et al.* 2009), while flowering and the fruiting occurs in the dry or in the dry-to-wet season transition and has been related to rainfall and temperature (Gottsberger & Silberbauer-Gottsberger 2006, Lenza & Klink 2006, Reys *et al.* 2013).

Deciduous species in seasonal environments shed their leaves after the beginning of the dry season and produce new leaves at the beginning of the next rainy season, while evergreen species are always presenting leaves, with a continuous replacement of senescent by new ones (Worbes *et al.* 2013, Alberton *et al.* 2014). Evergreen species may also completely leaf exchange in the middle of the dry season, when the new leaves flush and then the old leaves are shed thereafter. Brevi-deciduous tree species show a short leafless period of a couple of weeks during the dry season shed their leaves, when all the leaves are shed and then replaced by new leaves (Worbes *et al.* 2013).

Our results suggest a relationship between the minimum $\delta^{13}C$ values at the beginning and the end of the growth season, with maximum $\delta^{13}C$ values near the middle of the growing season and leaf flush and leaf fall, respectively. While high $\delta^{13}C$ values seem to be related to higher water use efficiency and the use of carbohydrates in the production of new leaves for photosynthesis maintenance, low $\delta^{13}C$ values seem to be related to leaf fall and low activity/dormancy of cambium, as well as to the high stomatal conductance in relation to photosynthesis ratio. Franco *et al.* (2005), analyzing tropical trees, found that deciduous species flush by the end of the dry season and present a decline of stomatal conductance in the same period. The peak of ACO_2 occurred at the peak of wet season, showing that the higher $\delta^{13}C$ is related to higher water use efficiency.

Pterodon pubescens is an import legume tree widely distributed in Brazilian cerrados. The anemochorous and brevideciduous species lose leaves in the middle of the dry season, and produces new leaves in the end of the dry season (Franco *et al.* 2005). The present study aims to understand the relationship among tree phenology, seasonal patterns of stable carbon isotopes and climate in *Pterodon pubescens*. This will be done by reconstructing a high-resolution stable carbon isotope series over the last eight years based on wood samples of three different trees. We will then link the seasonal patterns of $\delta^{13}C$ to climate and phenological activity aiming to elucidate climate effects on tree phenology. High-resolution stable carbon isotope series allow the understanding of how carbon and oxygen isotopes varied within each year and how the tree responded to the climate in a much more precise definition.

MATERIALS AND METHODS

Study area

This research took place in a Cerrado remnant of 260 ha, located at Fazenda São José da Conquista, municipality of Itirapina, São Paulo State, Southeastern Brazil (22º 10' 31.41"S; 47° 52' 26.1 3"W, 610 m of altitude). The remnant was formed about 30 years ago and presents a rectangular shape with sides facing the four cardinal points.

The vegetation is a cerrado *sensu stricto* (Coutinho 1978), a savanna vegetation characterized by the presence of small, leaning and tortuous trees, with irregular and twisted ramifications (Ribeiro & Walter 1998). The vegetation holds a discontinuous canopy of woody plants reaching six to seven meters high with a continuous herbaceous layer; the vegetation becomes denser at some parts with some trees reaching up to 12 meters high and reduced herbaceous vegetation (Camargo *et al.* 2011, Reys *et al.* 2013).

The climate of the cerrado is seasonal, with a warm and wet season from October to March and a dry and cold season from April to September. During all the studied period, from January 2005 to December 2012, the climate was similar among the years and confirmed the seasonal pattern described for the region (Camargo *et al.* 2011). The climatic data were provided by CHREA (Centro de Recursos Hídricos e Ecologia Aplicada), of University of São Paulo (USP), located 4 km far away from the study area.

The average of rainfall and temperature (maximum, mean and minimum) of the last 30 years in the Cerrado shows strong seasonality. The lowest values occurred at the middle of the year in the dry season, and the highest values at the end and beginning of the year, in the wet season. The highest amount of rainfall was observed in 2012, 2011, 2009, 1995 and 1991, and the lowest total rainfall was in the years of 2010, 2008 and 1994 (Fig.1). There was an increase of rainfall amount in 2011 and 2012 (Fig. 1).

The studied species

Pterodon pubescens Benth.

The species *Pterodon pubescens* (Fabaceae), and hereafter just *Pterodon*, occurs in the states of Minas Gerais, São Paulo, Goiás and Mato Grosso do Sul, and is found in both, the cerrado vegetation and in the transition to the semi deciduous forest (Lorenzi 2002). This wind dispersed species is deciduous and presents annual flowering and fruiting (Pirani 2009). In the study area, the species is one of the most occurring ones, being mostly concentrated in the edges of the fragment. *Pterodon pubescens* is an import legume trees widely distributed in Brazilian cerrados (Franco *et al.* 2005). The anemochorous and brevideciduous species loses leaves in the middle of the dry season, and produces new leaves in the end of the dry season (Franco *et al.* 2005). Franco *et al.* (2005) considers *P. pubescens* a deciduous species which stays leafless for 15±4 days.

Dendrochronology

We sampled between 2 and 4 wood cores per tree with a motorized auger from the main trunks of 6 individuals of *Pterodon* inside the phenology transects. The individuals selected to the sampling were the largest ones found within the population. The cores were drought naturally and polished with different grids of sandpaper (from 50 to 2000) (Urquiza 2008, Alvarado 2009, Soliz-Gamboa *et al.* 2010). All the parenchyma band rings were marked and measured using LINTab (Drobyshev *et al.* 2010) and TSAPWin program, to analyze the similarities between the radios and to compare the behavior of series as well as to verify which rings could be intraspecific markers (Locosselli *et al.* 2013). After the measurement, the growth rings had their widths processed by the program COFECHA (Soliz-Gamboa *et al.* 2010, Locosseli *et al.* 2013), which indicates the similarity between the individuals.

In order to build a relation between growth rings and phenological activity, we focused on the last 8 rings that represent the whole period of phenological data. The species *Anadenanthera peregrina var falcata* is brevideciduous and presents well defined growth rings indicated by a parenchyma band (Marcati *et al*. 2006).

Isotopes

We sampled between 2 and 4 wood cores per tree with a motorized auger from the main trunks of 6 individuals of *Pterodon* inside the phenology transects. The cores were drought naturally and polished with different grids of sandpaper (from 50 to 2000) (Urquiza 2008, Alvarado 2009, Soliz-Gamboa *et al.* 2010). All the parenchyma band rings were marked and the three individuals presenting the clearest ring pattern were selected and the cores of each individual were sliced with a diamond saw (max. 1000 µm thickness) and passed through a resin extraction. The last 8 years of each individual core slice were sampled using a high-resolution UV-laser-based microscopic (Schoallaen *et al.* 2013), which allows cutting a very precise and small sample within each ring (100-µm to 1000-µm thickness) (Schoallaen *et al.* 2013).

Between 10 and 40 cuts were made within each ring, depending on the ring width. Dimensions of the cuts were adjusted so that a yield was obtained between 180 to 200 µg of wood. Samples were weighed and packed in silver caps for the oxygen analysis and tin caps for the carbon one. Samples were analyzed at the GFZ – German Research Centre for

Geosciences in Potsdam, via conventional Isotope Ratio Mass Spectrometry (IRMS) coupled online to a combustion or pyrolysis furnace (Schoallaen *et al.* 2013).

Phenological observations

Phenological observations were recorded in 36 transects of 25 x 2 m, with at least 50 m of distance between them. Transects were distributed along the interior and the edge of East and South faces in the cerrado (see Camargo et al. 2011 for details). All individuals of *Pterodon pubescens* with circumference at the stem base larger than or equal to 3 cm were sampled, totalizing 18 individuals.

We conducted phenological observations at monthly intervals from January 2005 to December 2011 on the following phenophases: flower bud, anthesis or flowering, unripe fruiting, ripe fruiting, leaf flush and fall. We estimated the intensity of each phenophase using a semi-quantitative index of intensity ranging from 0 (absence) to 2 (peak), with an interval of 50%, (Opler *et al.* 1976, Morellato *et al.* 2000). The vegetative phenological activity pattern was analyzed based on the average of all individuals which shows the percentage of individuals with determined phenophase, for the three individuals with the isotope pattern and for the population of 18 individuals.

While leaf flush activity is positively influenced by maximum temperature (0,295), leaf fall is negatively influenced by minimum (-0.444), mean (-0.487) and maximum (-0.353) temperature, as well as by the monthly rainfall amount (-0.363).

Results

The average of vegetative phenological pattern from 2005 to 2012 also presented a strong seasonal pattern, with the peak of leaf flush activity in September following the leaf fall peak in July (Fig. 2). Similar to the population phenological pattern, the phenology of individual trees also presented leaf fall peak just followed by the leaf flush peak (Fig. 3). Our results suggest a relation between isotopes pattern, climate variables and phenological activity. Spearman correlation between monthly climate variables and phenological data showed that the leaf fall and flush were significantly influenced by rainfall and temperature.

Tree rings provide environmental information about climate, its seasonality and changes, and the intra-annual variations in $\delta^{13}C$, $\delta^{18}O$ and wood density are driven mainly by seasonal variations in climatic conditions. Dry periods, for example, are clearly recorded in the seasonal pattern of $\delta^{13}C$, when cambium activity is low or absent and the rings boundaries are formed (Helle & Schleser 2004). In this study, both carbon and oxygen high-resolution isotopes series presented a very clear seasonal pattern (Fig. 4). We detected two distinct phases in $\delta^{13}C$ pattern: an increase from seasonal minimum to a high (often maximum) value from the end of the ring to the start of the next ring; an often gradual decline or a rather constant $\delta^{13}C$ after reaching the high $\delta^{13}C$ at the start of the ring (Fig. 4). In some cases we observed first a slight rise in $\delta^{13}C$ followed by a decline (Fig. 4). The pattern of $\delta^{18}O$ also presents two distinct phases, inversely to the $\delta^{13}C$ pattern: a decrease from seasonal maximum to a low (often minimum) value from the end of the ring to the start of the next ring; an often gradual increase after reaching the low $\delta^{18}O$ at the start of the ring (Fig. 4). However, both patterns were clearer in some years than in other years, and the differences are probably resulting from the variation in samples number per ring.

In the ring boundary, which is a clear parenchyma band in *Pterodon*, we found the minimum and maximum values of $\delta^{13}C$ and $\delta^{18}O$ series, respectively (Fig. 4). The maximum value of $\delta^{13}C$ and the minimum value of $\delta^{18}O$ were both found in the middle of the ring, related to the wet season and highest values of climate variables (Fig.5).

The comparison between isotopes series, climate series, leaf flush and leaf fall activity showed that the growth ring boundary matches the lowest isotopic values for δ13C, in the dry season and peak of leaf fall (Fig. 6).

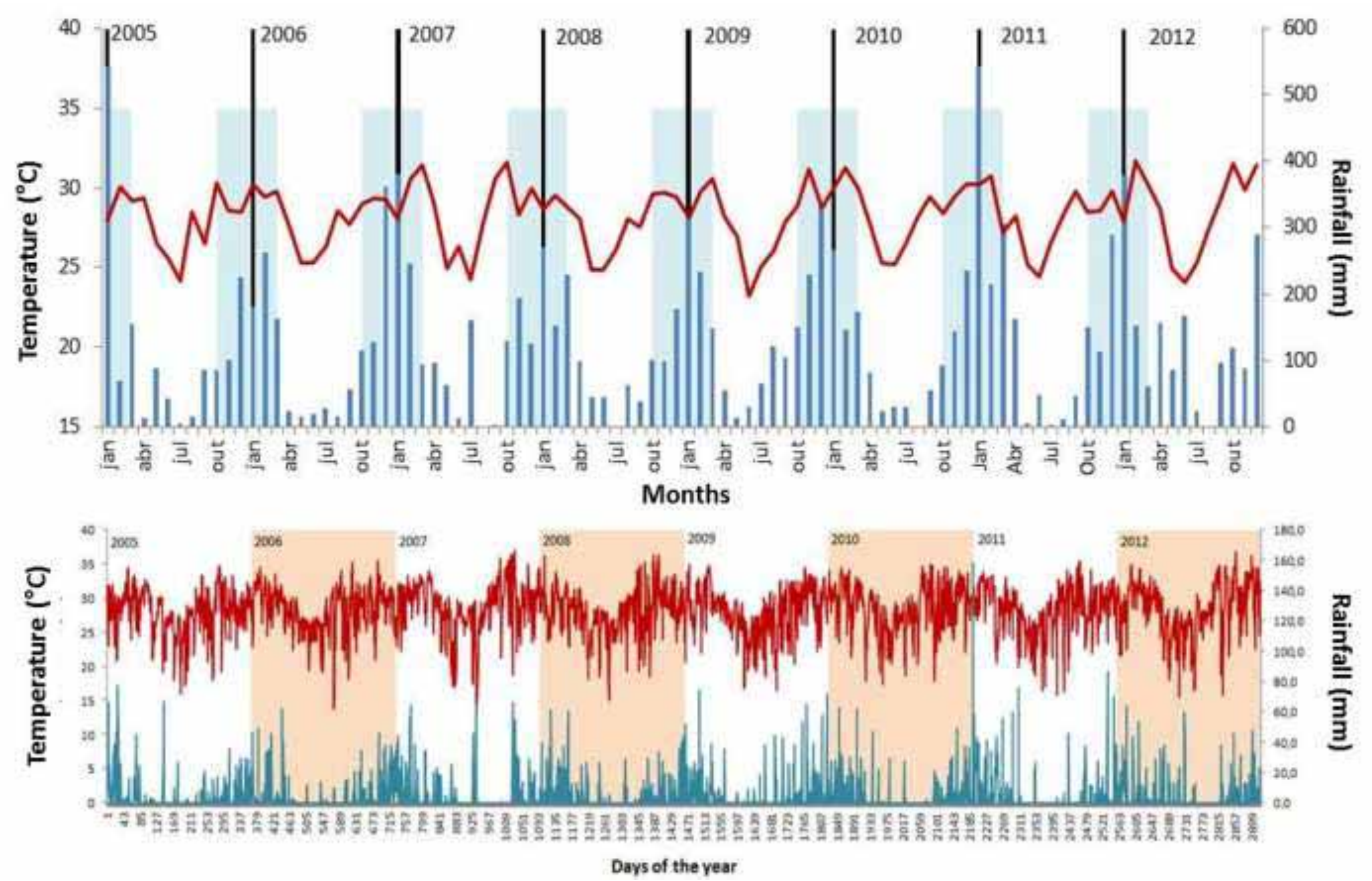

Fig. 1 –Maximum, mean and minimum temperature (red line) and rainfall blue bars sum at monthly (above) and daily (bellow) intervals 8 years in the area of Itirapina – SP, Brazil during eogth years. Blue shadows = wet season, yellow shadow demarks the years.

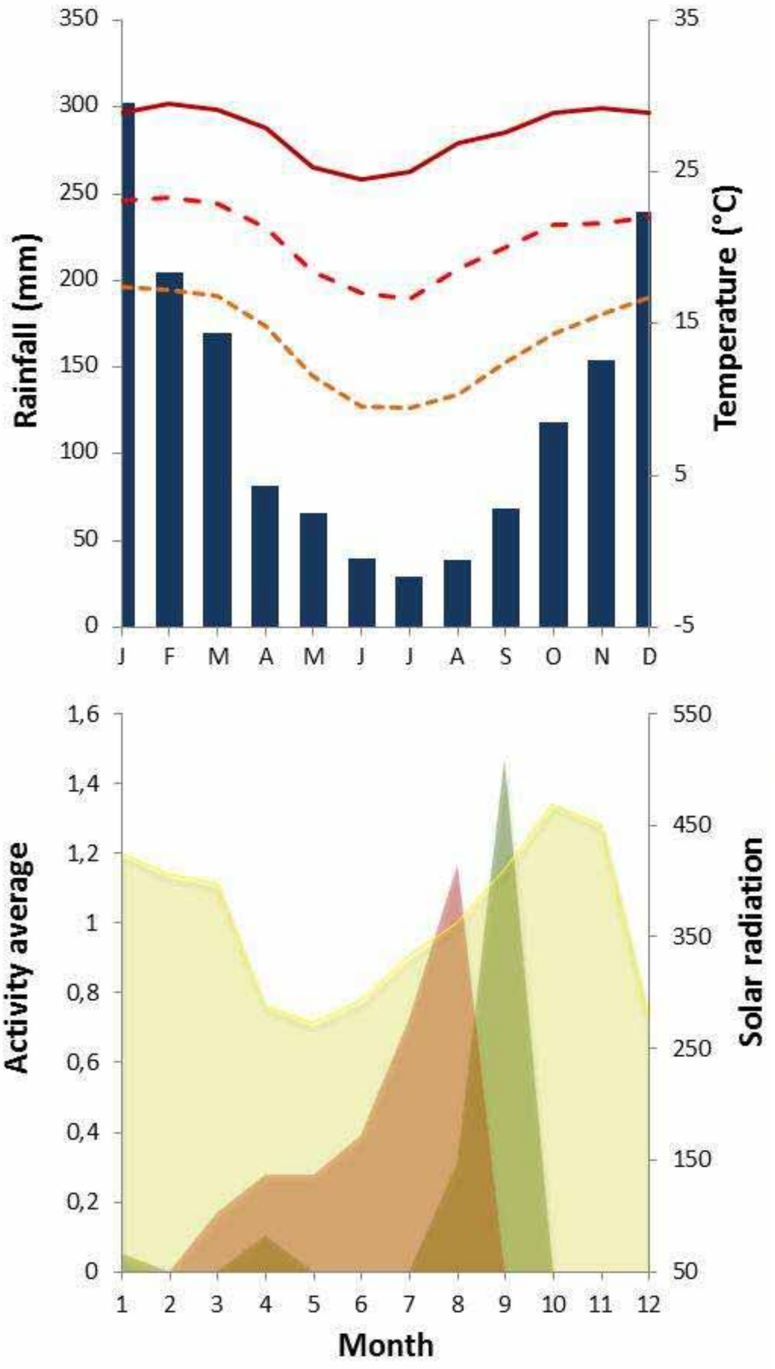

Fig. 2 – Total rainfall (blue bars), max medium and min temperatures (lines in the upper graph) and solar radiation average (lower graph – yellow line) for the last 24 years, and leaf fall (brown area) and leaf flush (green area) phenological activity average of the last 8 years (lower graph) in a cerrado .

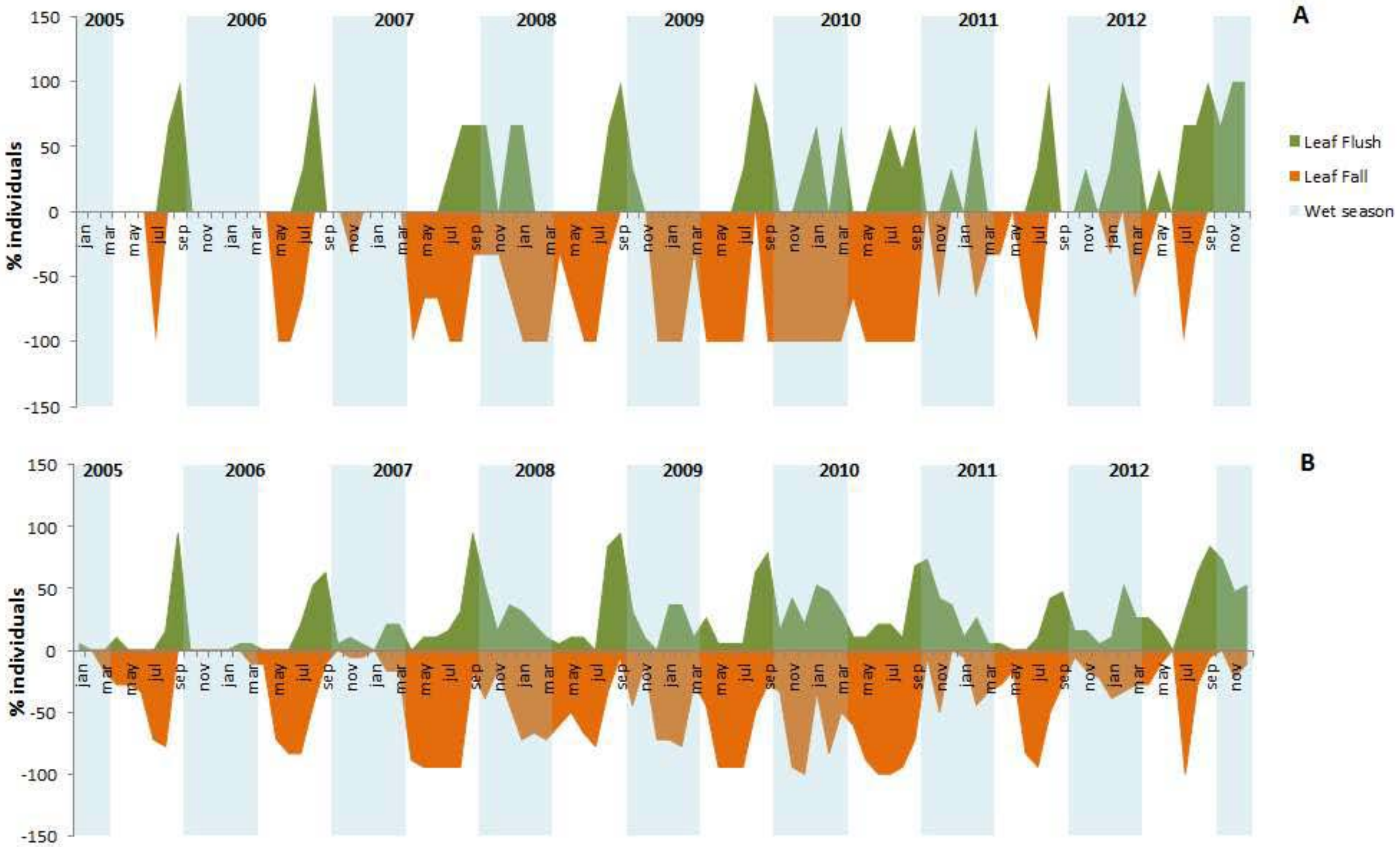

Fig. 3 – Vegetative phenological activity average (percentage of individuals presenting leaf flush and leaf fall activity) for (A) three individuals and (B) the entire population of 18 individuals of *Pterodon pubescens*, from 2005 to 2012 in a Cerrado, Southeastern Brazil.

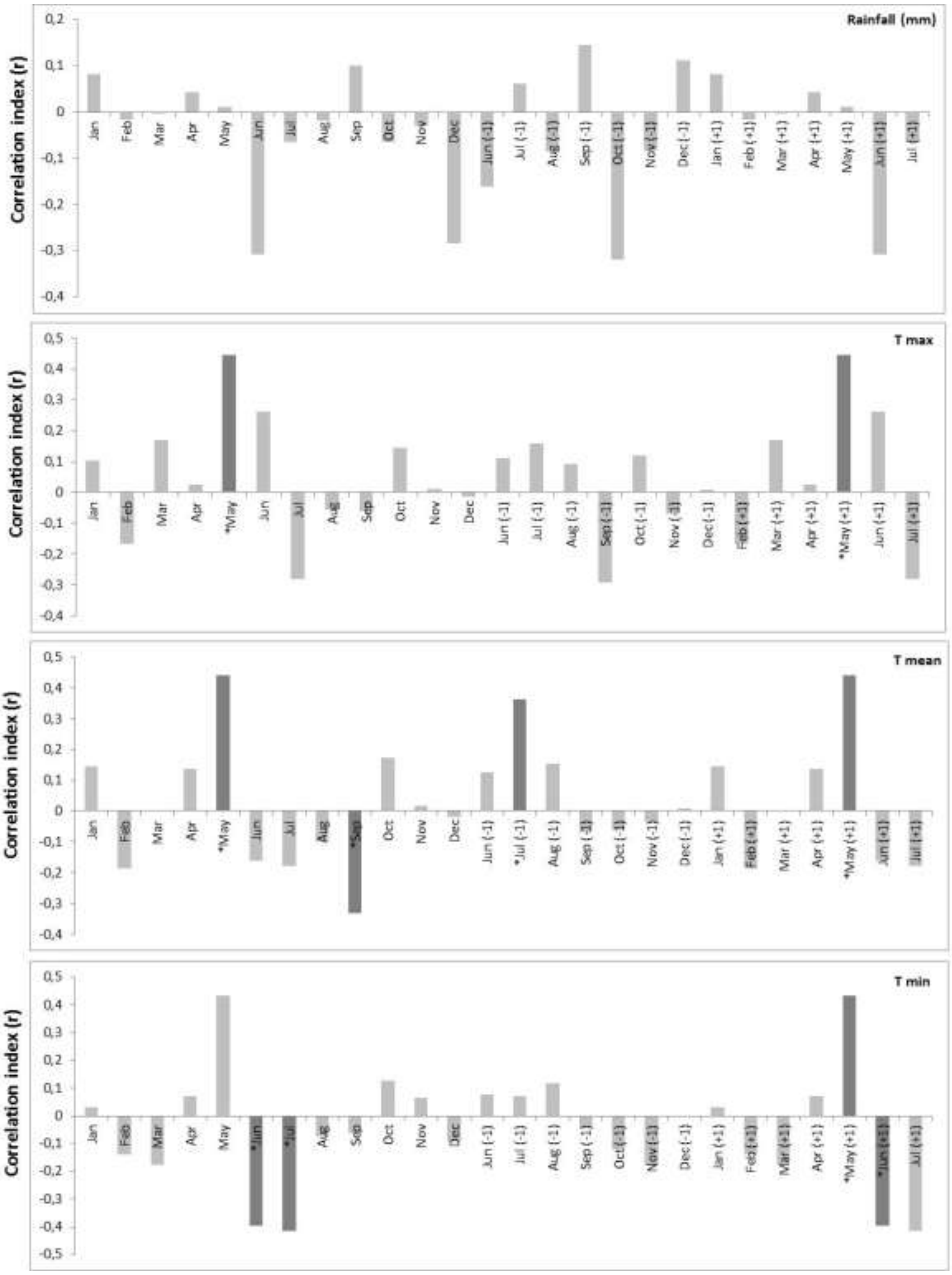

Figure 10 – Correlation between residual chronology from *Pterodon* and climate variables. Values marked with (*) shows significant results (<0,05).

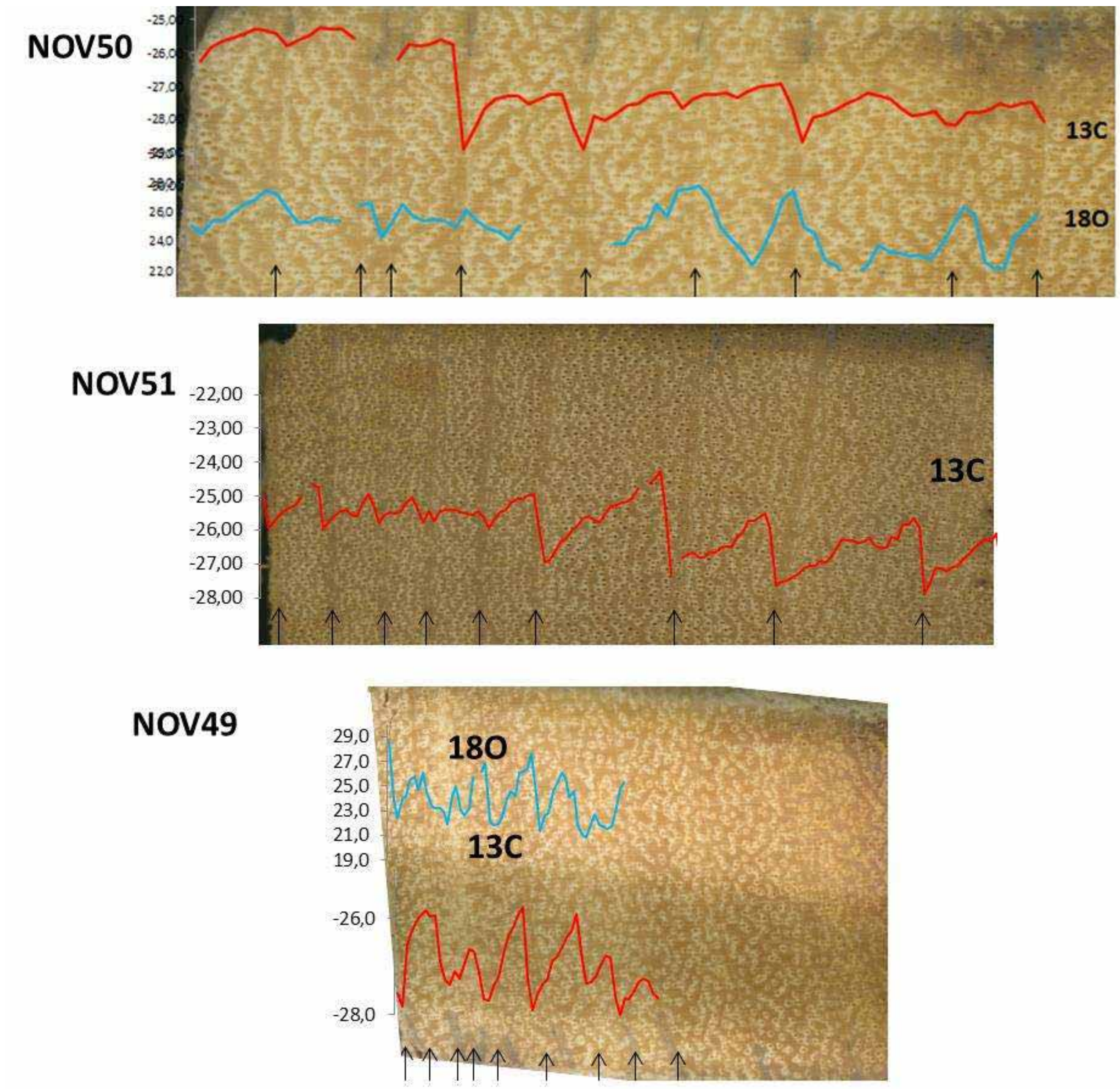

Fig. 4 – Variation on isotopes $\delta^{13}C$ and $\delta^{18}O$ starting in 2013 until 2005 plotted in the wood cores of three *Pterodon pubescens* trees (NOV49, Nov50 and NOV51) from cerrado, Southeastern Brazil.

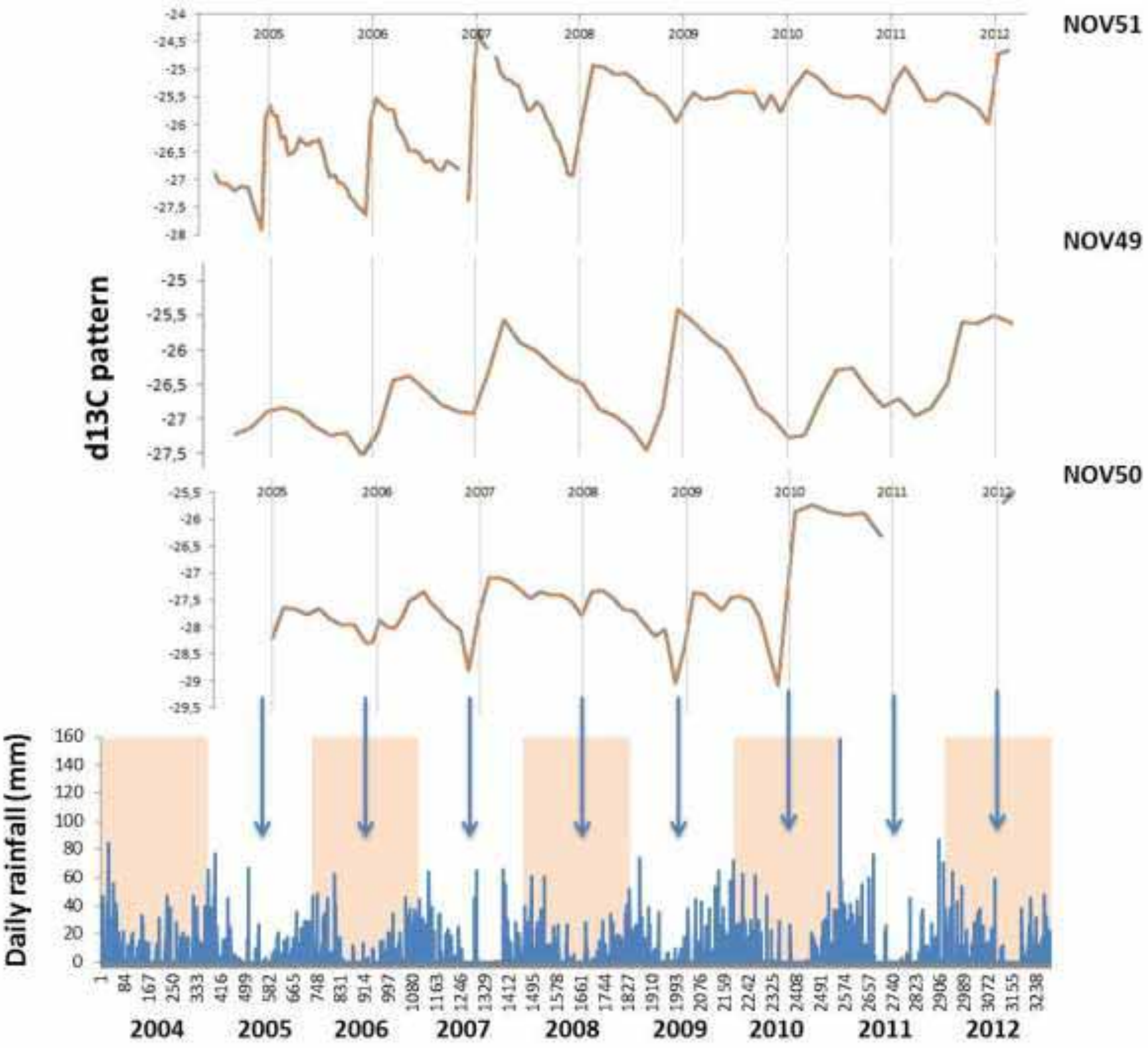

Fig. 5 – Comparison between NOV51, NOV49 and NOV50 (individuals) high-resolution isotope and daily rainfall, from 2005 to 2012, for *Pterodon pubescens* in a cerrado, Southeastern Brazil.

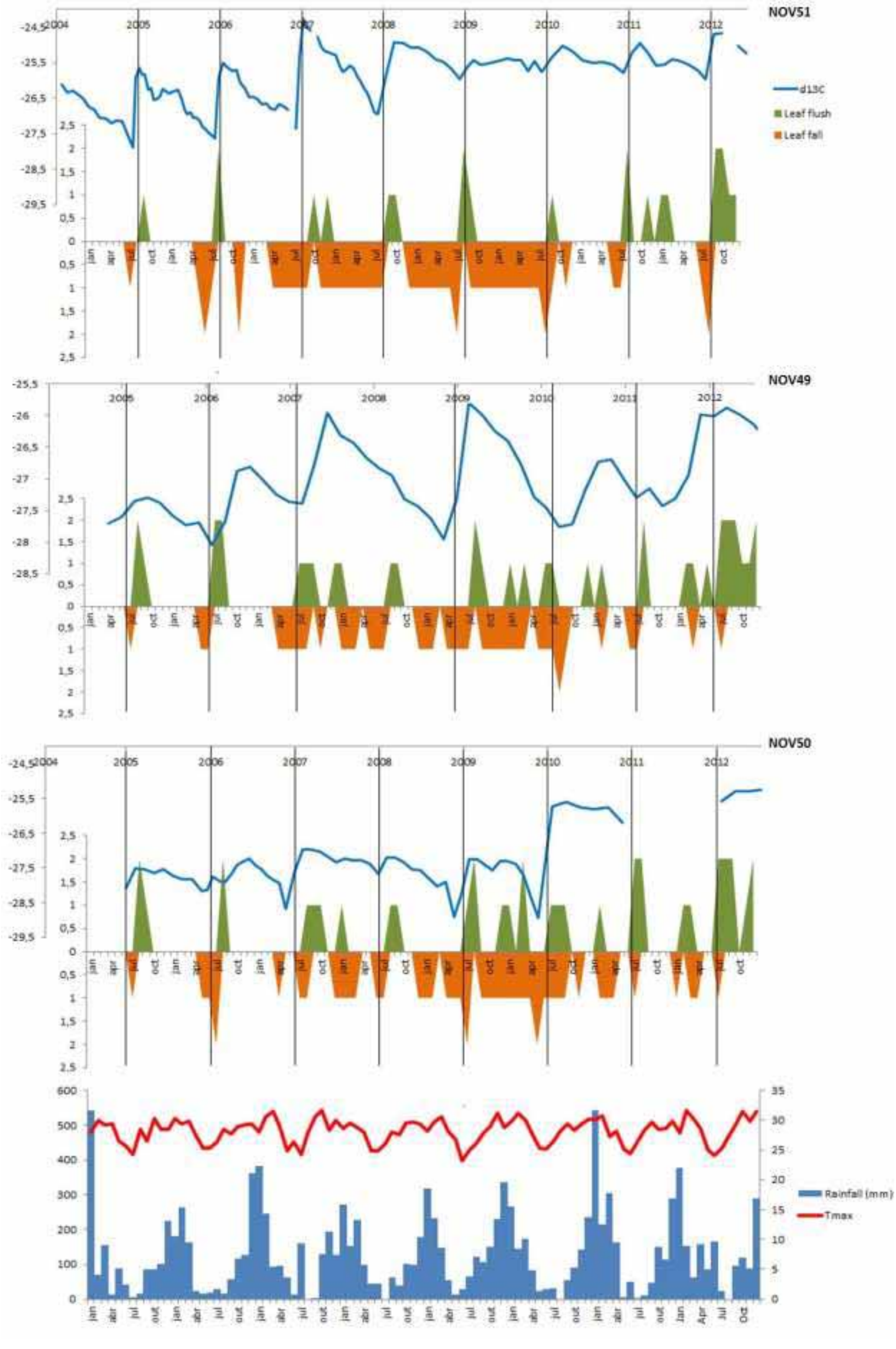

Fig. 6 – Individual vegetative phenology activity from 2005 to 2012 in a Cerrado, southeastern Brazil, in comparison with the isotope δ13C pattern and monthly climate.

Discussion

The studied species, *Pterodon pubescens,* is brevideciduous and presents a strong seasonal phenological pattern. The leafless period lasts for up to one month during the dry season. Losing leaves in the middle of the dry season and leaf flushing in the transition between the dry to the wet season allows the plant to reduce water losses during the winter, a period with low temperature and water availability and avoid herbivores attack (Franco *et al.* 2005).

Pterodon presented the highest δ^{18}O values at the ring boundaries (the parenchyma bands) and the lowest values in the middle of the growing season (Schoallen *et al.* 2013). Both studies showed a clear annual cycle, with annual wood formation starting with a parenchyma band, and δ^{18}Owd values that are similar to the δ^{18}Owd values at the end of the previous tree ring. The δ^{18}Owd maximum is followed by a decline to a seasonal minimum, typically in the middle of the growing season, before δ^{18}O marginally rises again in the next ring's boundary (Brienen *et al.* 2012). Although numbers of sub-sections per year varied because of the variation of each ring size, the pattern described is rather consistent and seems to follow the annual cycle of rainfall in the Cerrado. This pattern may be explained by the fact that δ^{18}O in wood at least partially comes from the variation in δ^{18}O in source water. Source water δ^{18}O concentration is inversely related to the rainfall seasonality with higher values during the dry season, and the lowest values in the middle of the rings (formed in the wet season) (Brienen *et al.* 2012). Thus, seasonal variation in δ^{18}O in wood is suggested to follow the seasonal variation in δ^{18}O of the source water and rainfall seasonality. The peak δ^{18}O in the ring boundaries of *Pterodon* corresponds to mid dry season, which is when the main leaf loss took place and when high temperatures increase water evaporation in the soil combined with low rainfall.

Our results corroborated Craig-Gordon and Roden models (Barbour 1997), showing a negative relation between oxygen isotopes and precipitation and relative humidity, meaning that higher evapotranspiration during the dry season leads to higher $\delta^{18}O$. On the other hand, higher atmospheric temperature, combined with lower rainfall, leads to higher evaporation of soil water increasing ^{16}O which leads to a lower $\delta^{18}O$ in the plant, seasonal pattern is also found in annual resolution from Brienen *et al.* (2012) research. During air-parcel transport, two processes affect the isotopic composition of water-vapor content in the plant (McCarroll & Loader 2004): First, heavy isotopes tend to condense more readily, and thus water vapor gets gradually more depleted during transport over land (water vapor loses relatively more of the heavier isotopes ($H_2{}^{18}O$) because of the classic Rayleigh distillation) (Brienen *et al.* 2012). The degree of total "rainout" of heavy isotopes depends on the fraction of water that is removed from a particular air parcel, and is thus generally larger during years with high amounts of precipitation along the air-parcel trajectory than during years with low precipitation (Brienen *et al.* 2012). Therefore, this mechanism leads to more depleted water vapor (more negative δ18O) at the end of the trajectory during wet years compared with dry years. Once climate variables present such strong influence on plants physiology, it is possible to understand climate effects on plant growth and also on their phenology (Brienen *et al.* 2012).

Intra-annual variation in isotopes showed that *Pterodon* presents a phase of rapid increase in $\delta^{13}C$ that coincides with the end of the last ring and the first wood of the new ring, crossing the parenchyma band. Just after the cambium low activity or dormancy combined to leaf fall activity peak, trees present an increase of $\delta^{13}C$ in growth rings wood, followed by the peak of leaf flush. The cambium low activity or dormancy during the dry season, in tropical dry forests, is the basic process and precondition for the formation of annual rings in the wood (Worbes & Raschke 2012), and shedding leaves allows the trees to avoid water loss, pattern also found by Worbes *et al.* (2013).

In trees, the d^{13}C value of leaf sugars is dominantly controlled by the ratio between ci ([CO_2] in plant mesophyll): ca (atmospheric [CO_2]). If ci is high relative to ca, stomatal conductance is much higher than the rate of photosynthesis, which leads to a strong carboxylation discrimination against ^{13}C, producing low δ^{13}C values (McCarroll & Loader 2004). If the stomatal conductance is high, it is expected to have relatively more CO_2 in the mesophyll than when the conductance is low, meaning that the moisture is higher. This leads plants to have a higher setting of ^{12}C. On the other hand, if it is too dry, the stomata will be more closed. In addition, when there is more rain and moisture, plants are able to increase photosynthesis activity, which increases the rate of assimilation, favoring even more the rate of ^{12}C. In drier times, the stomata are closed and thus the assimilation rate also decreases, reducing fractionation in favor of the ^{12}C and leading to a higher δ^{13}C value, which is found at the beginning of each ring in *Pterodon* cores, also corroborating the pattern found by Helle & Schleser (2004). Once δ^{13}C pattern in *Pterodon* was found to be related to leaf fall (lowest values of δ^{13}C) in the end of the ring and leaf flush (highest values of δ^{13}C) in the beginning of the ring, we may suppose that leaves just after flushing are less able to discriminate carbon than young formed leaves (Taiz & Zeiger 2006), once just flushed leaves maintain the stomata closed and present a lower photosynthetic activity, which leads to higher values of δ^{13}C. Young leaves are not in their highest photosynthetic activity, however, are probably source of carbon, which leads to a decrease in δ^{13}C values. Therefore, while just flushed leaves behave as a drain of carbon, once they are flushing and being formed, young leaves become a carbon source once they open the stomata and start photosynthesis activity (Taiz &

Zeiger 2006), and dynamic which seems to explain the relation between the isotope and the phenological patterns found in *Pterodon* growth rings.

Vegetative phenology is suggested to be related to high-resolution isotope patterns, specially leaf fall and the deciduousness of species (Helle & Schleser 2004). Gulbranson & Ryberg (2013) described the differences between the deciduousness of temperate trees and their growth rings isotopic patterns. They found that the patterns of $\delta^{13}C$ values in growth rings differ between evergreen and deciduous trees. *Pterodon pubescens* was a brevideciduous species and presented a pattern with sharply rising $\delta^{13}C$ at the start of a ring and a negatively decline along rings formation. Although all rings present some degree of $\delta^{13}C$ increase, there is a lot of variation between the years. The individual NOV51 up to 2008 and NOV49 (2007 and 2008), for example, showed a very typical pattern of maximum $\delta^{13}C$ at the start of the ring (from wood with high ^{18}O thus formed during the dry season) followed by a near linear decline. This pattern is a typical "idealized deciduous pattern", such as those presented by Gulbranson & Ryberg (2013) (Fig. 7), who studied isotope patterns of temperate species.

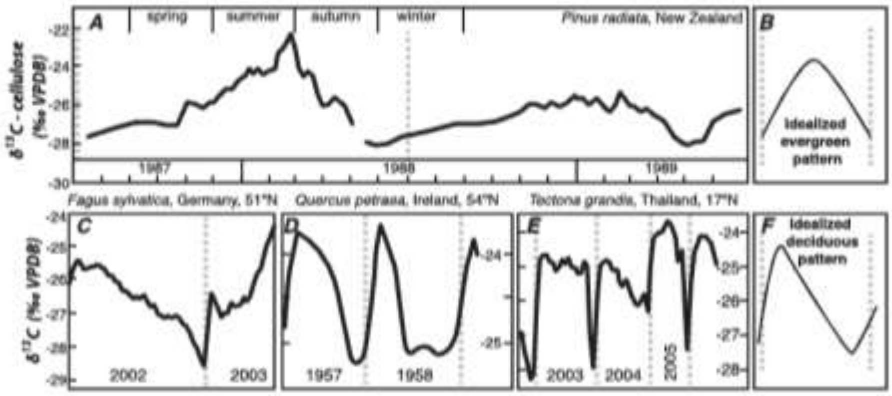

Fig. 7 - Examples of high-resolution carbon isotope ratios in tree rings of extant plants. Original figure published in GULBRANSON AND RYBERG (2013).

We suggest a relationship between growth rates and leaf flush in *Pterodon*, since leaf flush peak coincided with the highest δ^{13}C values, just after the minimum values that might be related to the dry season and the consume of carbohydrates to leaf production. High values of δ^{13}C are found when the rate of stomatal conductance is lower than the ratio of photosynthesis, leading to the decrease of internal CO_2 concentration and less discrimination against ^{13}C (McCarroll & Loader, 2004). While maximum δ^{13}C values were related to the bud burst, the minimum δ^{13}C-values in the mid of the dry season are similar to the minimum δ^{13}C-values in autumn, pattern also found by Helle & Schleser (2004). Comparing isotopes pattern between leaves and wood, Helle & Schleser (2004) found that the highest δ^{13}C-values observed in the foliage occur at highest growth rates, at leaf flush stage, while the highest δ^{13}C-values of tree rings are probably related to the highest cambial activity of the wet season (Fig. 8). The δ^{13}C-peaks observed in beech's young foliage and early wood probably occur simultaneously, since bud swelling and cambial activity start at about the same time.

Helle & Schleser (2004) also divided the isotope pattern found in growth rings in three different stages. The first section is when isotope pattern reaches the maximum δ^{13}C values, correlated to the leaf flushes and explained by the use of carbon reserve to produce the leaves. The highest values of δ^{13}C found in this study and in Helle & Schleser (2004) study, correlated to leaf flush, were also found by Damesin & Lelarge (2003).

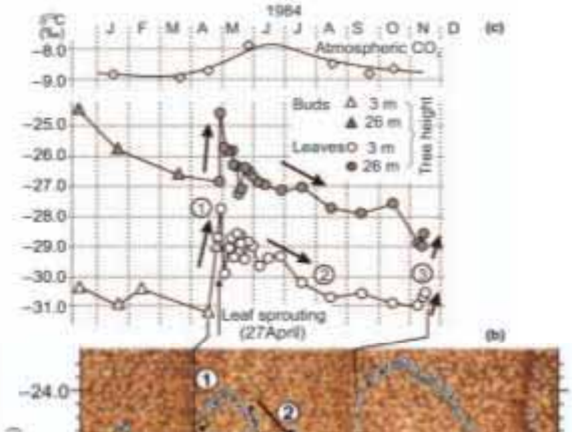

Fig. 8 – Isotope pattern from Helle & Schleser (2004) showing the *relations*hip between leafing and the stages of isotopes through growth rings.

Plants use carbohydrates as a carbon source in order to supply winter respiration and build new tissues in the absence of photosynthesis during leafless period (Taiz & Zeiger 2006). This response is considered a post-photosynthetic isotope effect (Helle & Schleser, 2004; Offerman *et al.*, 2011). *Pterodon* growth rings form increasingly positive δ13C values across the ring boundary (point of cambium low activity or dormancy), with maximum δ^{13}C values in the earlywood, while the early wood-latewood transition contains progressively negative δ^{13}C values (Fig. 9). This asymmetric trend is displayed by deciduous trees in contrast to evergreen trees, and may be explained by the impact of whole new leaf growth in the spring as a result of the use of carbohydrate storage from previous growing seasons (Barbaroux & Breda, 2002).

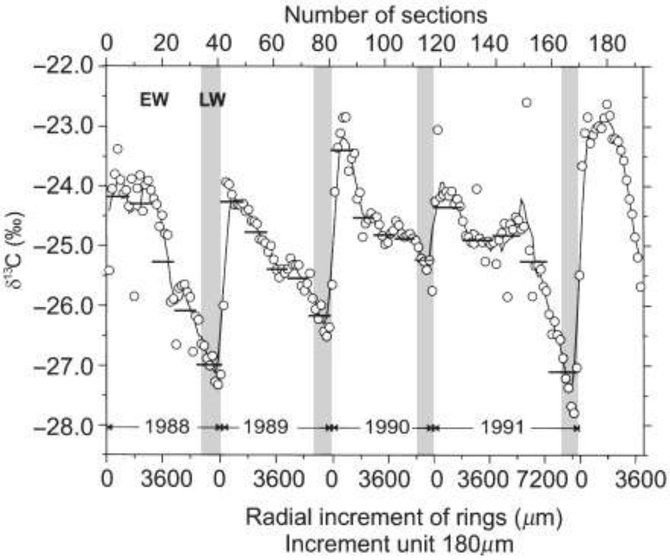

Fig. 9 – Helle & Schleser (2004) results for high-resolution isotope pattern analysis from *Populus nigra* growth rings.

High respiration rates are found when leaves start to flush and early-wood begins to be produced, in order to meet the energy requirements of the tree in this period of fast growth (Teskey & McGuire 2002). In early spring, the proportional period of the year correspondent to start of tropical wet season, branch parenchymatic tissues associated with the phenological activity of leaf flush, suffer an increase in metabolic activity that leads to a branch and stem respiration rise independently of xylem temperature (Helle & Schleser, 2004). When maximum δ^{13}C enrichment of emerging leaves and highest cambial activity in the stem is reached, maximum respiration rates are supposed to occur (Helle & Schleser, 2004).

The use of the carbon stored during the dry season for wet season growth may lead to an enrichment of recently formed biomass. Trees growth switches from storage material to current substances synthesized in photosynthesis when young leaves begin to export photosynthates, as well as the cambial activity starts to decrease (Helle & Schleser 2004). The observed decline of δ^{13}C in biomass is justified by the gradual incorporation of photosynthates into recently formed cell wall material, once trees use both stored and currently produced carbohydrates for growth maintenance, often at the same time (Helle & Schleser 2004). Recurrent phenophases activity such as flowering, fruiting and leaf flushing, as well as short-term increase of cambial activity, probably influence on the seasonal progression of δ^{13}C in tree rings (Helle & Schleser 2004).

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Final considerations

This research brought important contributions to the understanding of the relationship between phenology, the pattern of trees growth and its stable isotopes of carbon and oxygen under the influence of climate and its seasonality in Cerrado. Both species were promising and under a clear influence of seasonality. Although they presented different relationships with climate, since *Anadenanthera peregrina var falcata* showed to suffer influence of precipitation and temperature on its growth pattern, while *Pterodon pubescens* showed a relationship only with temperature, *Pterodon pubescens* was more promising for studies of dendrochronological reconstruction and climate, since it has only a clear ring-shaped line which is the marginal parenchyma band, forming more well-defined rings. This ease of recognition and analysis of growth rings allowed greater clarity in the study of the isotopes of carbon and oxygen, and therefore, this was the species chosen for the second chapter research. Analyzing the stable isotopes of carbon and oxygen from *Pterodon pubescens* growth rings, we found a clear seasonal pattern, allowing us to understand the relationship between climate, the species growth, its phenology and physiological processes in response to climate and its changes. The analysis of high-resolution stable isotopes of carbon and oxygen had never been held in a Cerrado area before and was proved to be extremely reliable and promising for a better understanding of the effects of climate and its changes on the physiology, phenology and ecology of tropical species.