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Original Article

Leaf phenology and wood formation of white cedar trees (*Melia azedarach* L.) and their responses to climate variability

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Abstract

This research aimed to investigate the response of leaf phenologies and wood increments of *Melia azedarach* L. on climate variability. The visual estimation indicated the leaf flushing in January-July, the leaf maturation in January-November, and the leaf abscission in May-June and October-December. Monthly wood investigations of the inside bark diameters (IBD) indicated an annual-ring formation with the wood increment in February-November and the dormancy in December-January. The outside bark diameter (OBD) exhibited growth variations with phases of slow increment in September-October, shrinkage in December-February, and fast increment in March-August. The relationship among monthly climates, leaf phenologies and wood increments, indicated the significant correlations of the soil moisture and the abundances of mature dark green leaves on the IBD, while the OBD was fluctuated due to the direct effect of the IBD and the indirect effect of the soil moisture and mature dark green leaf abundances.

Keywords: annual-ring, leaf phenology, monthly wood increment, *Melia azedarach*, path analysis (PA)

1. Introduction

Climate variability is the variations from the mean state of the climate statistics while climate change refers to any change of climate over time (Ramamasy & Baas, 2007). The variability mainly has impacts on human health (Tunde *et al.,* 2013), economic values (Bruggeman *et al.,* 2011) and environments (Flantua *et al.,* 2015). Several techniques and

mathematic models are applied to investigate the past climate for the longer periods in order to better understand and helpful to make mitigation plans for climate variability and changing management.

Although, the climate data recorded using meteorological instruments is reliable and illustrates the highest accuracy, the data series is too short to investigate the past climate fluctuation and difficult to construct the precise climate modeling. Other proxies such as ice core from glaciers and the polar ice caps (Alley, 2000), fossil and pollen records (Lezine *et al.,* 2011), sediments (Zolitschka *et al.,* 2015) and tree-ring data (Shi *et al.,* 2014) are valuable for measuring past climates in order to support the predictive climate

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modeling. Many publications of the past climate reconstructions were done by using tree-ring data, especially in temperate and timberline zones, which tree species forming annul rings were widely distribution (Fischer & Neuwirth, 2013; Wahl *et al.*, 2012), but the studies in the tropics (between 23°26′14.1″ N and 23°26′14.1″ S) was still limited.

Since 1900s, it was unlikely to see tree-ring research zone toward the tropics due to the abundant distribution of indistinct or absent ring species in this area with the unclear seasonality. Worbes (2002) reported that the tropical tree-ring analysis was successfully done by Berlage who constructed annual-ring chronology of teak (*Tectona grandis*) in Indonesia and it could be significantly related to the fluctuation of precipitation. In Thailand, teak and two species of pines (*Pinus kesiya* and *P. merkusii*) had defined as distinct annual-ring species, which were successfully done for palaeoclimate reconstruction (Buckley *et al.*, 2007; Pumijumnong & Wanyaphet, 2006).

However, the knowledge of periodic wood formation in other tropical tree species in Thailand is still rare and raises the question of which species forming the true annual-ring and responding to climate are not yet solved. In order to improve the limitations of the tropical tree-ring studies, it is essential to explore the information of which tree species is potentially available for climate-growth responses and annual-ring analysis. Fritts (1976) explained the general growth model of the trees as the relationship of climate and annual growth periodicity in terms of phenology and wood increments. By using a modified method of cambial wounding with Mariaux's windows (Mariaux, 1967), a technique to detect the annual growth periodicity in the wood, the mature white cedar trees (*Melia azedarach* L.) illustrating a distinct growth ring, which the information of periodic wood and annual-ring formations still lacked and the potential for annual-ring analysis involved climate variability was not studied yet, were selected to investigate annual-ring formation association with other factors described in the general growth model of the trees in order to study the potential for climate-growth responses.

Therefore, this study aimed to examine leaf phenologies and monthly wood increments of *M. azedarach* in order to support their growth responses on climate variability. An additional objective was to investigate wood anatomy of *M. azedarach* in order to identify annual-ring boundaries for serving tree-ring analysis. This proved to be an opportunity to improve the limitation of the tropical tree-ring studies and expand the network of climate reconstruction by using *M. azedarach* due to the extensive distribution of this tree species in several regions around the world.

2. Materials and Methods

2.1 Study site

The mature *M. azedarach*, which were planted with non-silvicultural management, were provided at the Wang Nam Khiao Forestry Student Practicing Station (WNKFSPS) of the Faculty of Forestry, Kasetsart University in Nakhon Ratchasima province, northeastern Thailand (Figure 1). This site was selected, not only for the occurrence of the mature white cedar trees, but for the study locations proximity Sakaerat Environmental Research Station (SERS) where the
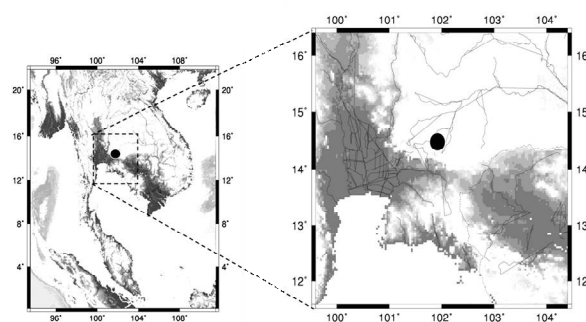

Figure 1. Study site: the black dot indicated the Wang Nam Khiao Forestry Student Practicing Station (WNKFSPS) at Pak Thong Chai district, Nakhon Ratchasima province, northeastern Thailand.

useful climate data of rainfall, relative humidity and temperature have been carefully recorded since 1969.

2.2 Methods

Six mature and healthy *M. azedarach*, which had straight trunks with symmetrical crowns and were located on the good drained area, were selected. During September 2009-August 2010, leaf phenologies which was generally divided to four phases of leaf flushing (LF), mature light green leaves (MLL), mature dark green leaves (MDL) and leaf abscission (LA) were monthly investigation by using the visual estimation with a pair of binoculars (O'Brien *et al.*, 2008; Vitasse *et al.*, 2009). Within the crown cover of each tree, all leaf pheno-phases were estimated and scored from 0 to 5 as a bare crown (score = 0), 1–20% (score = 1), 21–40% (score = 2), 41-60% (score = 3), 61-80% (score = 4) and greater than 80% (score = 5). In each month, soil samples were also randomly collected for 3 points at 5, 10, 15, 20, 25, and 30 cm soil depths by using a soil auger and soil moisture contents were then analyzed by using the gravimetric method (Black, 1965).

Monthly wood increments which were measured from outside and inside bark diameters were also investigated. A modified manual band dendrometer with 0.1 mm accuracy of vernier scale was installed at breast height (130 cm) of each selected tree and the circumference change was monthly recorded to determine the outside bark diameter increment (OBD) coincided with their leaf phenologies (Buckley *et al.*, 2001). Using a modified method of cambial wounding (Mariaux, 1967), cambial zones were monthly injured by using a modified knife in order to investigate the inside bark diameter increments (IBD) and cell differentiation. A year later, twelve cambial wounds from each tree were then extracted and carefully polished on the transverse surfaces using several grades of abrasive papers until the injured zones were prominently visible with clean and smooth surfaces.

From the first cambial wounding in September 2009 until the last marking in August 2010, the IBD increments were monthly measured from the distance between the adjacent cambial wounds by using the Velmex measuring system with the nearest 0.001 mm accuracy association with the stereo microscope (Palakit *et al.*, 2015). Total increment was then calculated from the summation of all monthly wood increments. Micro-sections of total wood increment were also

conducted by using a sliding microtome at a thickness between 10-15 μm to investigate the anatomical characteristics and to obtain the marked cells of annual-ring boundaries.

To explain the effect of climate variability on growth of *M. azedarach*, monthly climate data of rainfall, temperature, relative humidity and soil moisture contents with the multi-collinearity removal by using the technique of principle component analysis (PCA) (Hu & Bentler, 1999; O'Brien, 2007) were hierarchically related to leaf phenological characteristics and monthly wood increments of IBD and OBD by using the application of multiple linear regression, namely path analysis (PA), which illustrated both of the direct and indirect effects of the climate data on tree growth in terms of leaf phenologies and monthly wood increments.

3. Results and Discussion

3.1 Climate data

Climate data in 1969-2010 derived from SERS was analyzed and could be classified to 2 types of the wet (March-November) and the dry (December-February) seasons. The mean temperature and the total rainfall in the wet season were 27.1 °C and 1,043.0 mm, respectively. In the dry season, the temperature declined and was lower than the mean of the wet season for 3.3 °C as similar as the rainfall data in the dry season which was totally recorded only 31.9 mm. Due to the influences of the southwest and the northeast monsoons, the nine months of the wet season could be divided into two parts of March-July and August-November with the total rainfall amounts for 470.4 and 572.5 mm, respectively. The highest temperature occurred at the beginning of the wet season in April for 29.2 °C and the lowest temperature could be recorded in December for 21.9 °C (Figure 2).
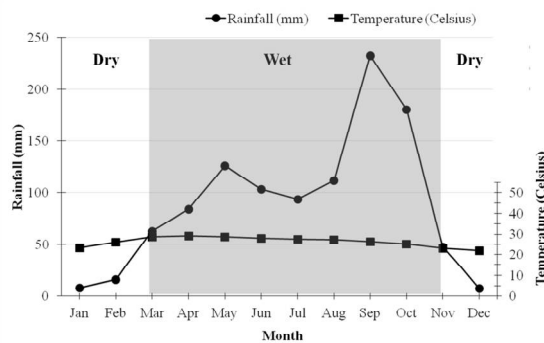

Figure 2. 41 years climate data of monthly rainfall and temperature derived from the Sakaerat Environmental Research Station (SERS). Gray box indicate the wet period in March to November.

3.2 Monthly growth investigation

Leaf pheno-phases of six *M. azedarach*, coded MA01 to MA06, were invested in September 2009-August 2010. Leaf flushing (LF) emerged in January-July, while small amounts of mature light green leaves (MLL) could be rarely found in several months and could be abundantly found in June. Mature dark green leaves (MDL) were firstly found in

February after leaf flushing occurring in January and was abundantly found in April-May. In June, the abundances of MDL and MLL were equivalent due to the declined MDL and the increasing MLL. MDL re-abundantly found in August-October. Leaf amounts of MDL decreased since November and almost completely disappeared in December. Leaf abscission (LA) rarely found in May-June and October-December, while all leaf pheno-phases were rarely found in the dormant periods of December-January (Figure 3).
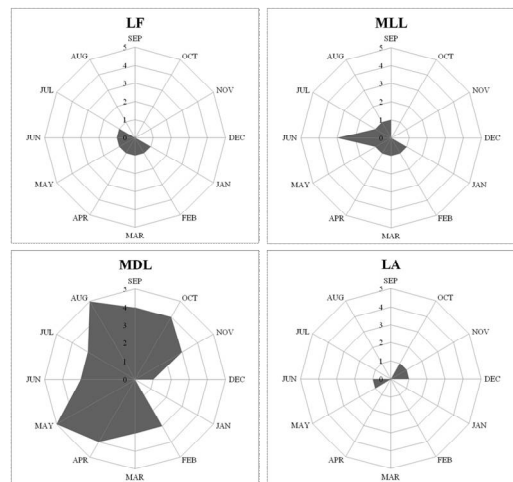

Figure 3. Leaf phenology of *M. azedarach* during the investigated periods including leaf flushing (LF), mature light green leaves (MLL), mature dark green leaves (MDL) and leaf abscission (LA). The observed scores are ranging from 0 to 5.

Monthly wood increments measured from the outside bark diameter (OBD) were also recorded and the mean of total wood increment was 1.05 cm. The averages of cumulative and monthly OBD increments of all selected trees were shown in Figure 4. Cumulative and monthly growths of these white cedar trees fluctuated with phases of the slow increment in September-October and the shrinkage in December-February. Later, the fast increment occurred in March till the end of August.
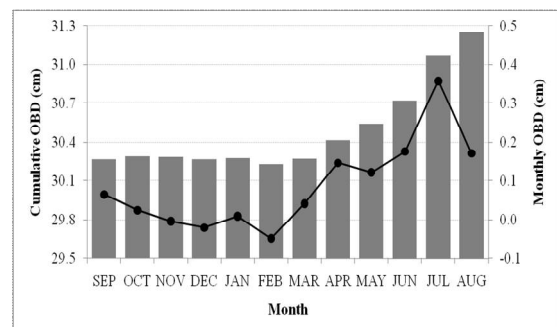

Figure 4. Cumulative and monthly increment of outside bark diameters (OBD) in *M. azedarach*. Gray box and black line indicate cumulative and monthly OBD, respectively.

Inside bark diameter (IBD) increments of *M. azedarach* gently increased since the transitional period of the dry and the wet periods in February and the maximized

growth occurred in July and August for 2.46 and 2.34 mm, respectively. In September-November, monthly IBD increments gradually declined to 1.61, 1.55 and 0.39 mm, respectively. Growth dormancy occurred in the dry period of December and January (Figure 5). The total wood increment measured from monthly IBD increments was 1.56 cm.
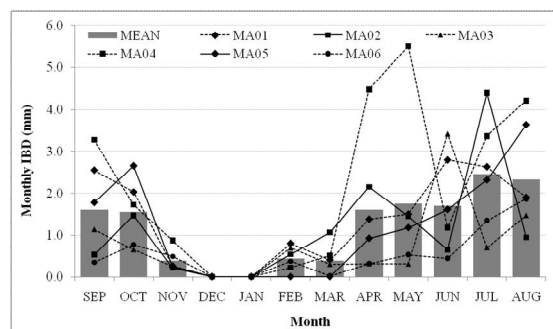

Figure 5. Inside bark diameter (IBD) increments of six *M. azedarach* and their monthly averages (gray bar) during the investigated periods

Monthly wood increments of *M. azedarach* which were detected by the cambial wounding technique initiated association with the leaf flushing at the beginning of the wet season. The cessation of wood increment occurred when leaf abscised following by the leaf absent in the dry season, especially in December till January. Leaf abundances and wood increments rapidly increased and maximized in the wet season. It could be claimed that the lower rainfall during the winter strongly reduced wood increments, while the wet condition during the rainy season induced wood increments (Lisi *et al.,* 2008; Marcati *et al.,* 2006, 2008).

Cufar *et al.* (2008) also found the leaf unfolding following by the immediate reactivation of cambial cells and the three-fourths of the annual-ring width was formed in the first half of the total cambial active period. During the period of cessation, the products of photosynthesis were probably accumulated as reserves in storage tissues (Cufar *et al.,* 2008). In case of the evergreen tree species, wood formations of *Aglaia odoratissima* and *Hydnocarpus ilicifolia* were not related to leaf phenologies and the seasonal climate variation (Palakit *et al.,* 2015).

Leaf abscission of *M. azedarach* occurred at least a month each year and the abundances of mature dark green leaves (MDL) directly induced inside bark diameter (IBD) increments. As similar, Venugopal and Liangkuwang (2007) found cambial activities and annual rhythm of xylem production of *Dillenia indica* Linn. after the sprouting of new leaves and buds for 15 days. It was also similar to the study of Cufar *et al.* (2008) who illustrated leaf unfolding of *Fagus sylvatica* in SE central Europe associating with the reaction of cambial and wood increments. At La Selva Biological Station, Costa Rica, deciduous trees also showed a significant relationship between stem increment and leaflessness, while evergreen trees found no relationship (O'Brien *et al.,* 2008).

After the first wounding was done at the beginning of September, rates of monthly wood increments of *M. azedarach* gradually decreased and small vessels were found until the end of November (Figure 6a). The IBD increment in these three months was 3.55 mm (22.79% of the total IBD increment) and the average vessel diameter was 0.12 mm. The
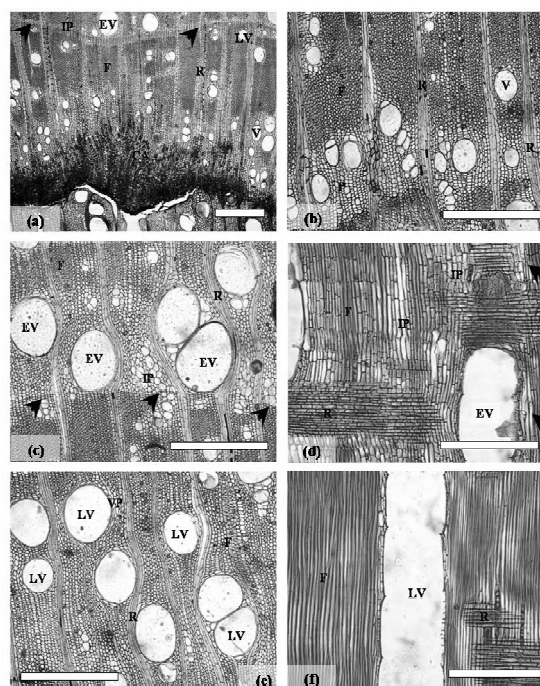

Figure 6. Microscopic features of *M. azedarach* wood: (a-b) callus cells (CA) and parenchyma (P) from cambial marking effect; (c-d) earlywood vessel (EV) associated with initial parenchyma (IP); (e-f) latewood vessel (LV) associated with vasicentric parenchyma (VP). F = fibers and R = ray cells. White bands indicated the length of 500 μm. Arrow head indicated the initial parenchyma.

dormant growth occurred for two months in December-January and the IBD increment was re-stimulated in February for 0.44 mm (2.82%). In this month, the initial banded parenchyma was found associated with large vessel diameters (0.23 mm) (Figure 6c). The appearances of the largest vessels indicated that the dormancy was gone and tree growth had started as the beginning of annual-ring formation of the next growing season. Until the end of August, vessel size diameter mildly declined to 0.21 mm.

As similar as the most of deciduous tree species, *M. azedarach* could be defined as a ring porous species which vessel sizes in earlywood zones are normally larger than those in latewood zones (Boura & Franceschi, 2007; Ohashi *et al.,* 2009). Although, *M. azedarach* illustrated the ring porous characteristic, *M. dubia* which is the indigenous species of southern India illustrated the diffuse porous characteristic (Saravanan *et al.,* 2013). In order to classify the annual-ring formative species and identify their annual boundaries, wood anatomical studies should be done to indicate the most common and most identifiable characteristics including presences of marginal parenchyma, vessel diameter variation in earlywood and latewood, and the thickest wall fiber in latewood (de Pernia & Melandri, 2006; Marcati *et al.,* 2006; Roig *et al.,* 2005).

After annual-ring identification, the next procedure is to justify the ring formation occurring once a year. Using the cambial wounding boundaries, *M. azedarach* formed marginal parenchyma associating with the larger vessels at the beginning of the growing season and illustrated the smaller

vessels at the end of the growing season. It was similar to several studies which tree species producing annual-rings were indicated by marginal parenchyma and thick walled latewood (Lisi *et al.,* 2008; Marcati *et al.,* 2008). Other techniques, including the counting of growth rings corresponded with the documented tree age (Chowdhury *et al.,* 2008) and radiocarbon dating (Pearson *et al.,* 2011) were also successful for annual-ring identification.

However, cambial wounding also generated the anomalous cell which disturbed the analysis of periodic wood and annual-ring formation. A thin banded parenchyma formed closed to the injured point and looked similar to the initial banded parenchyma but the variation of vessel diameters which were gradually decreased from earlywood to latewood was the useful characteristic to identify annual-ring boundaries. The initial of annual-ring, which the largest vessel diameter formed and was found association with the marginal parenchyma, was shown in Figure 6c, while the banded parenchyma formed from the effect of cambial marking was shown in Figure 6b.

3.3 Climate-growth relationship

Leaf phenology and monthly wood increment data were related with climate data of relative humidity (RH), rainfall, maximum temperature (Tmax), minimum temperature (Tmin) and mean temperature (Tmean). Soil moisture contents derived from the differences between wet and dry weights of soil samples from several soil depths, including 0-5 (SM5), 5-10 (SM10), 10-15 (SM15), 15-20 (SM20), 20-25 (SM25) and 25-30 (SM30) cm, were also examined the relationship with leaf phenologies and monthly wood increments. Climate data and soil moisture contents illustrated the significant linear relationship, called multi-collinearity, which the tolerance (TR) was less than 0.20 and/or the VIF was equaled to 5 and above (O'Brien, 2007). Therefore, the technique of principle component analysis (PCA) was used to convert a set of possibly correlated variables into a set of linearly uncorrelated variables, called principal components (PCs). These correlated variables were converted to 3 components of climatic data based on the criteria of Eigenvalues ≥1.

The first component was related to soil moisture contents in all soil depths and was re-named as '*Soil Moisture*". The second component related to temperature in both of Tmax, Tmin, and Tmean and was re-named as "*temperature*". The last component related to rainfall and RH data which was re-named as "*precipitation*". Each component was not significantly correlated with other components and could explain the cumulative variance of all selected climatic factors for 95.145% (Figure 7).

Path analysis (PA) was applied to analyze both direct and indirect effects of all climate components on leaf phenologies and monthly wood increments. The testing of the constructed model (Figure 8: Model A), which monthly wood increments of *M. azedarach* responded climate variability, indicated the suitable and passed the overall model fit examination as similar as the model of leaf phenologies responding climate variability (Figure 8: Model B).

The sequential model A could be explained that both of the soil moisture component and the mature dark green leaf (MDL) directly induced monthly wood increments of *M. azedarach*, while the occurrences of the MDL were not significantly related to all climate components. Soil moisture component and the MDL could explain the increment of the white cedar wood for 61%. Outside bark diameter (OBD) increments detected by manual band dendrometers was directly influenced by inside bark diameter (IBD) increments and indirectly influenced by soil moisture component and the MDL through the IBD. These direct and indirect influences could explain the changing of the OBD increments for 64%.

For model B, the temperature component directly induced the occurrences of the leaf flushing (LF) and the mature light green leaf (MLL), while the precipitation component induced the leaf abscission (LA) and the LF. The corporation of temperature and precipitation components could explain the occurrences of the LF for 73%, while the changing of the temperature could explain the variation of the MLL for 42% and the changing of the precipitation components could explain the variation of the LA for 35%.

From the path diagram in Figure 8, it could be hierarchically explained that the increasing soil moisture content associated with the abundances of the MDL stimulated the development of wood. The OBD of *M. azedarach* was increased due to the increment of the IBD which was stimulated from the variation of the soil moisture content and the MDL as described above. Although the MDL was not significantly related to all climate and soil moisture

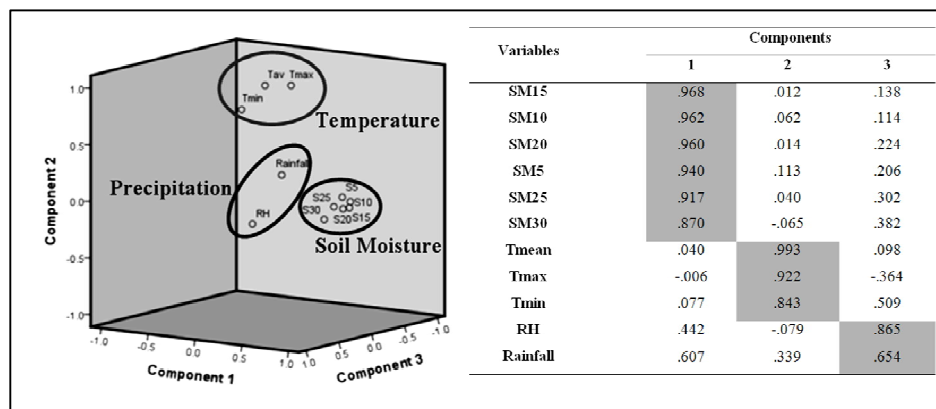| Variables	Components		
	1	2	3
SM15	.968	.012	.138
SM10	.962	.062	.114
SM20	.960	.014	.224
SM5	.940	.113	.206
SM25	.917	.040	.302
SM30	.870	-.065	.382
Tmean	.040	.993	.098
Tmax	-.006	.922	-.364
Tmin	.077	.843	.509
RH	.442	-.079	.865
Rainfall	.607	.339	.654

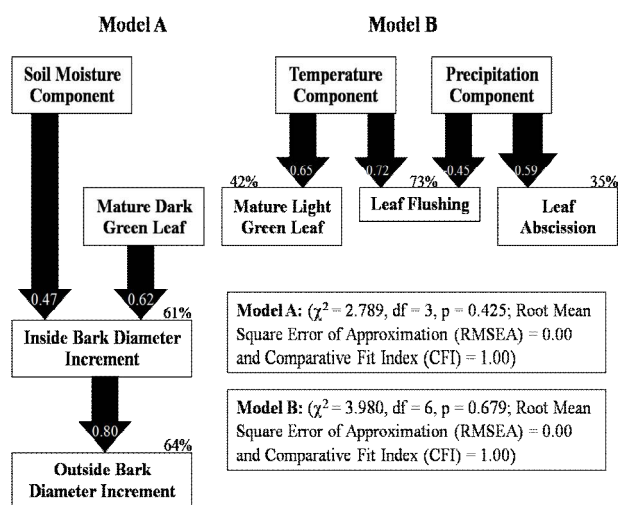Figure 7. Three components of "temperature", "precipitation" and "soil Moisture".

Figure 8.   Path diagrams: factors affecting monthly tree growth of *M.azedarach*: model A - factors affecting monthly wood increment; model B - factors affecting leaf pheno-phases. The correlation coefficient (r) and the coefficient of determination (r^2) were shown at the arrowhead and above the boxes, respectively.

data, its abundance occurred due to the continuous development of the LF and the MLL which were induced by the temperature and precipitation components.

Soil moisture component illustrated the direct response to the IBD increments and indirect response to the OBD increments through the increments of IBD. However, the major factor affecting the IBD increments of *M. azedarach* was the abundances of the MDL. It was different with the evergreen tree species which the abundances of the MDL occurred throughout the year and the formation of monthly wood increments was not significantly related to leaf abundances (Palakit *et al.,* 2015). Palakit *et al. (*2015) also found the insignificant correlation between climate and monthly wood increments of *A. odoratissima* but moisture was significantly related to wood increments of *H. illicifolius*. Lisi *et al.* (2008) found trunk increment dynamics of 24 tree species from a seasonal semi-deciduous forest of southeast Brazil corresponding to seasonal changes in precipitation, while Borchert (1999) suggested the seasonal rainfall driving leaf phenology, cambial growth and wood increment of deciduous trees.

Although wood increments of *M. azedarach* were not significantly related to the temperature and precipitation components, several researchers suggested temperature influencing cambial activities and wood increments of other tree species such as *Araucaria angustifolia* (Oliveira *et al.,* 2009), *Picea abies* (Nocetti and Romagnoli, 2008), *Pinus sylvestris* and *Betula spp.* (Schmitt *et al.,* 2004).

The OBD increments of *M. azedarach* directly and positively related to the IBD increments of xylem elements. Although, soil moisture component was not directly induced the OBD increments, it still induced the OBD increments through the MDL occurrences and the IBD increments. Several studies explained the OBD increments relating to the xylem growths of new cell formation and development, association with the bark swelling and shrinkage due to water content from the moisture and the precipitation (Bräuning *et al.,* 2008; Gruber *et al.,* 2009). In order to avoid the effect of bark swelling and shrinkage on the seasonal rhythms of xylem growth during the short investigated periods, Makinen *et al.* (2008) recommended the utilization of the cambial wounding technique instead of the installation of dendrometer bands.

4. Conclusions

Leaf phenology and the monthly wood formation of *Melia azedarach* were conducted to investigate the responses on climate variability. All leaf pheno-phases, except the mature dark green leaves (MDL), generally related to the fluctuation of temperature and precipitation. The occurrences of MDL mainly induced the formation of xylem elements, called the inside bark diameter (IBD) increments, which illustrated the annual distinct ring formation. In each annual-ring, the largest vessels association with the marginal parenchyma occurred at the beginning of the wet season following the declined vessel sizes to the smallest vessels at the end of the wet season. Growth dormancy occurring in the dry period was indicated by the leaf abscission, the unchanged IBD and the declined outside bark diameter (OBD) increments. The soil moisture component induced both of the IBD and OBD increments, while temperature and precipitation were not shown a significant relationship with IBD and OBD increments. For the further studies, the increment cores of the mature *M. azedarach* growing in the natural forest shall be collected to investigate the long-term periodic growth and its potential on the environmental and climate variability. Additionally, *M. azedarach* growing on several areas shall be studied to confirm the discovery of this research. The weather station shall also be constructed to record the precise climate for climate-growth studies.

Acknowledgements

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