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COMMUNICATION

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VEGETATIVE AND REPRODUCTIVE PHENOLOGY OF *AQUILARIA MALACCENSIS* LAM. (AGARWOOD) IN CACHAR DISTRICT, ASSAM, INDIA

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Abstract: *Aquilaria malaccensis* Lam., a threatened tree commonly called agarwood, is emerging as one of the most promising commercially important aromatic species in the world. This paper presents the findings on the vegetative and reproductive phenology of *Aquilaria malaccensis* from the secondary tropical evergreen forest in Cachar district, Assam. The effect of tree phenology and the influence of seasonal drought and environmental variables, especially temperature and precipitation, on various phenophases such as leaf initiation, leaf-fall, flowering, and fruiting were investigated. For this, a quantitative assessment was made at 15-day intervals by tagging 35 trees over a period of two years. Seasonal influence on the phenology of different phenophases was correlated with environmental variables and Spearman's rank correlation coefficient was employed. Leaf initiation was positively correlated with temperature ($r_s=0.694$, $p<0.05$), while leaf-fall was negatively correlated with temperature ($r_s=-0.542$, $p<0.05$) and rainfall ($r_s=-0.521$, $p<0.05$). Flowering ($r_s=0.713$, $p<0.01$; $r_s=0.713$, $p<0.01$) and fruiting ($r_s=0.721$, $p<0.01$; $r_s=0.775$, $p<0.01$) were positively and significantly influenced by temperature and rainfall. The study suggests that temperature and rainfall were major determinants of the vegetative and reproductive phenology of *A. malaccensis*, and any changes in these variables under expected climate change phenomenon may have a profound effect on phenophases of this threatened tree species.

Keywords: Agarwood, evergreen, phenology, tropical secondary forest.

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Author Contribution: Research design (AKD, AJN and BB); field study, data analysis and photography and manuscript writing (BB); manuscript correction and revision (BB, AKD and AJN). All authors approved the final version of the manuscript.

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INTRODUCTION

Phenology of tropical trees has attracted much attention nowadays from the point of view of conservation of tree genetic resources as well as forestry management, and for a better understanding of the ecological adaptations of plant species and community-level interactions. The study of tree phenology provides knowledge about the pattern of tree growth and development as well as the effects of environment and selective pressures on flowering and fruiting behaviour (Zhang et al. 2006). Phenology of vegetative phases is important, as cycles of leaf flush and leaf-fall are intimately related to processes such as growth, plant water status, and gas exchanges (Reich 1995). Studies of phenology are of great importance in determining the temporal changes that constrain the physiological and morphological adaptations in plant communities for utilization of resources by fauna (van Schaik et al. 1993). The sunshine hours, temperature, and annual precipitation have been recognized as the main environmental indications for leafing and flowering in the tropics. In many evergreen species, leaf flush and flowering occur close in time on the same new shoot. Variation in flowering time relative to vegetative phenology, induced by a variety of factors (significant rain in winter/summer, decreasing or increasing photoperiod, or drought-induced leaf-fall), results in a number of flowering patterns in tropical trees (Borchert et al. 2004). Phenological processes are significant constituents of plant fitness, since the time and duration of vegetative and reproductive cycles affect the capability of a plant species to establish itself in a given site (Pau et al. 2011). Singh & Kushwaha (2005) suggested that climate change forced deviations in the length of the growing period, and competition among species may change the resource use patterns in different species. Global climate change may force variations in timing, duration, and synchronization of phenological events in tropical forests (Reich 1995).

Although a few research works have addressed the population dynamics of the species in homegardens, northeastern India (Saikia & Khan 2013), an attempt has been made to study the phenology of *A. malaccensis*, which could contribute towards the conservation and management of the species, considering its almost extinct status in the wild (Anonymous 2003). Therefore, the present study is aimed to assess the phenological behaviour of *Aquilaria malaccensis* in a secondary tropical evergreen forest to understand the response of climatic variables and the periodicity of seasons.

MATERIALS AND METHODS

Study area

The phenological study was conducted in a secondary tropical evergreen forest located at Sonachera in Cachar District of Assam, northeastern India (Fig. 1). Secondary forests are those forests that regrow largely through natural processes after significant anthropogenic disturbance of the primary forest vegetation at a single point in time or over an extended period of time, and place prominently a major change in tree diversity and/or species composition with respect to nearby original forests on similar sites (Chokkalingam & de Jong 2001). The studied secondary tropical evergreen forest covers an area of 5 hectares. The geographical location of the study site is 24.36°N latitude & 92.44°E longitude and altitude range from 73 to 102 m.

Topographically, the area is characterized by typical terrain and hillocks that harbour diverse biological diversity. The climatic condition of the study area is subtropical, warm, and humid. Maximum precipitation occurs during the months of May to September, which is

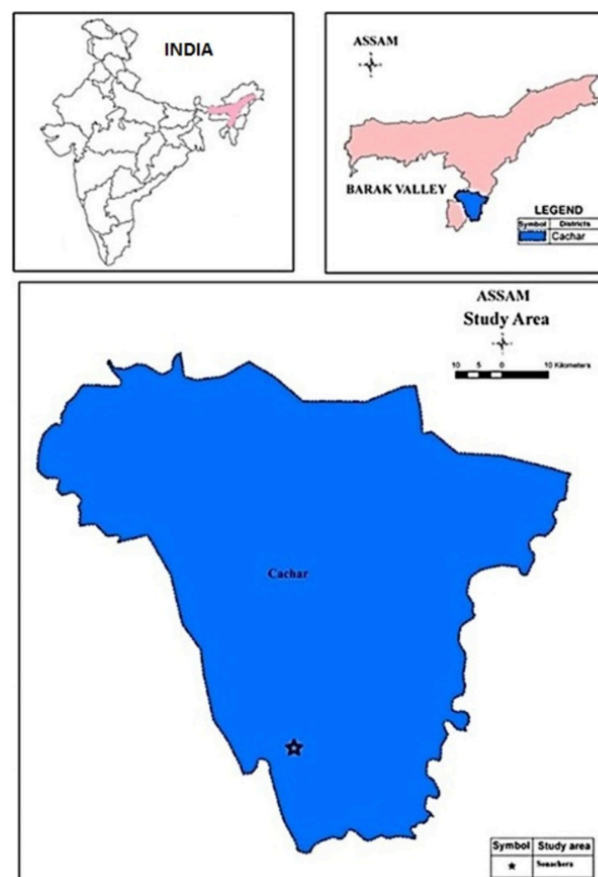


Figure 1. Map showing the location of the study area in Cachar District, Assam.

mainly controlled by the south-west monsoon season. The mean annual rainfall of the study area during the study period (2013–15) was about 2055.8mm, most of which (94%) occurred during April–September. The mean annual minimum and maximum temperatures were 19.9°C and 31.6°C, respectively (Fig. 2). The mean annual relative humidity was recorded at 75.9%.

The forest is categorized as “Cachar Tropical Evergreen Forest (Champion & Seth) (reprinted) (2005) (1B/C3)” type dominated by *Chrysophyllum roxburghii*, *Maniltoa polyandra*, *Memecylon celastrinum*, *Mesua floribunda*, *Palaquium polyanthum*, and *Pterospermum lanceifolium*. The selected secondary forest site is more than 35 years old. The secondary forest of this region is relatively unexplored and harbours a rich plant diversity.

Study species

Aquilaria malaccensis Lam. (Thymelaeaceae) is one of the most important species of commercial products in the world and is valued for its fragrant resinous dark-coloured wood known in trade as agar. Agarwood is formed by a complex plant-microbial interaction of a parasitic ascomycetous fungus known as *Phaeoacremonium parasiticum* (Ng et al. 1997). Phytogeographically, the distribution of *A. malaccensis* comprises the region of India, Myanmar, Sumatra, Peninsular Malaysia, Singapore, Borneo, and the Philippines (Chua 2008). In northeastern India, it occurs mostly in the foothills of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura (Saikia & Khan 2012a). In Upper Assam, the species is commonly cultivated in home gardens in association with other valuable plants for its high commercial demand (Saikia & Khan 2012c). The bark of the plant is also used as raw material for preparing a writing paper called ‘Sanchi pat’ for writing religious scripts (Nath & Saikia 2002). The agarwood is imported locally and exported internationally due to its wide use in the incense and perfume industry (Manohara 2013). Agarwood oil is a valuable component and is used as a digestive, sedative, analgesic, antiemetic and antimicrobial agents in tradition medicine (Cui et al. 2013). In the past few years, large-scale harvesting has caused rapid depletion of the stock in the natural forests. According to the IUCN Red List, the species is globally Vulnerable A1cd (Ver 2.3; IUCN 2017) and has been included in The World List of Threatened Trees (Oldfield et al. 1998). The species is also listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 1994).

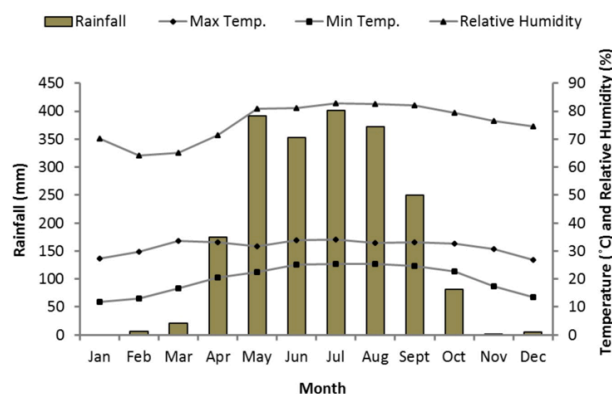


Figure 2. Ombrothermic diagram for the study period (2013–2015). Data collected from Tocklai Tea Research Association, Silcoorie, Silchar.

METHODS

A total of 35 individuals with girth size ranging from 12.7 to 81.7 cm were selected for phenological observations. The selected trees were marked with a metal tag for phenological observations. Using binoculars, phenological observations were made on leaf initiation, leaf-fall, flowering, and fruiting of the marked individuals. Phenological observations were based on phenological score: zero for no phenophase, one for less, two for moderate, and three for high (Broadhead et al. 2003). Detailed observations were carried out at 15-day intervals over a period of two years from February 2013 to January 2015.

Data analyses

Data analysis was performed using statistical software (MS Excel 2010) and (SPSS 21) version. Spearman rank correlations were performed to investigate correlations between monthly phenophase activity and environmental variables such as temperature and rainfall following (Zar 1984). The duration was classified as short (<2 months), intermediate (2–5 months), or extended (>5 months) based on the mean number of months in which the phenophase occurred (Luna-Nieves et al. 2017). Circular statistical analyses were performed to determine whether the vegetative and reproductive phenophases were homogeneously distributed throughout the year. For this purpose, months were converted into angles with intervals of 30°, and then the mean angle or mean date (α), the circular concentration (r), and the circular standard deviation (SD) were calculated. (α) indicates the time (month) of the year in which the largest number of individuals of a given species presented a phenophase, while (r)

indicates the degree of dispersion or concentration of the observations (Zar 1984). To determine the significance of the angle, a Rayleigh test (z) was used. There is a seasonality in the phenophases if the average angle is significant. The intensity of circular concentration (r) values varies from 0 (phenological activity uniformly distributed throughout the year) to 1 (phenological activity concentrated in a particular period of the year) (Morellato et al. 2010). We used the program ORIANA 3 (Kovach 2007) for these analyses. The meteorological data of the study area are presented in (Fig. 2).

RESULTS

Leafing activity

A. malaccensis initiated leafing during the pre-monsoon period (March–April) and continued up to a warm monsoon period throughout the favourable season (July–August) (Fig. 3A). The degree of circular dispersion or concentration ($r=0.99$) indicates that the phenophase leaf initiation was concentrated in a particular period of the year (Fig. 5). The phenophase leaf initiation was seasonal (Rayleigh Z , $p < 0.01$), and it occurred once a year. Peak leaf initiation was observed during March–April (Fig. 4). During the years 2013–14 and 2014–15, temperature registered its influence on leaf initiation significantly whereas rainfall displayed its impact in 2014–2015 (Table 1). The combined effect of temperature and precipitation, rather than their individual effects, more strongly influenced leaf initiation. Leaf-fall occurred during November–March with peak fall during January–February (Fig. 4). Rainfall and temperature presented a negative slope in correlation with leaf-fall due to decreasing day length and rainfall (Table 1).

Flowering

Flowering occurred during April–June (Fig. 3C & 4). The degree of circular dispersion or concentration ($r=0.97$) indicates that the phenophase flowering was concentrated in a particular period of the year (Fig. 5). The flowering phenophase was intermediate (Rayleigh Z , $p < 0.01$), and the open flower lasted one month. The duration of flowering phenophase ranged from 30 to 85 days with an average duration of 58.05 ± 6.35 days during 2013–14 and 32–90 days with an average duration of 61 ± 6.37 days during 2014–15, and it varied greatly among the individuals with a coefficient of variation (C.V. % = 20.62). Flowering was significantly influenced by temperature and rainfall while in one-month lag period

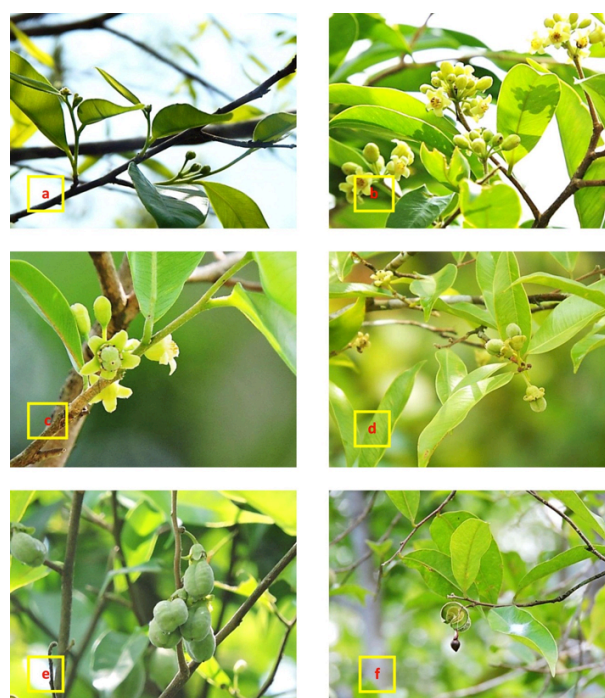


Image 1. a - Flowering initiation, b - Flowering inflorescences, c - Fruiting initiation, d - Young fruit, e - Mature fruit, f - Dehiscent fruit. © Birkhungur Borogayary

Table 1. Phenological patterns of *A. malaccensis* in relation to corresponding month and one month lag period of temperature and rainfall in Cachar District, Assam

Phenophases	Environmental factors			
	Spearman's rank correlation coefficients (r_s)			
	Temperature ($^{\circ}\text{C}$)		Rainfall (mm)	
	2013-14	2014-15	2013-14	2014-15
Leaf initiation	0.694*	0.861**	(0.251 NS)	0.528*
	(0.125 NS)	(0.340 NS)	(0.427 NS)	(0.199 NS)
Leaf-fall	-0.542*	-0.661*	-0.521*	(-0.444 NS)
	(-0.293 NS)	(-0.358 NS)	(-0.174 NS)	(-0.358 NS)
Flowering	0.713**	0.684*	0.713*	0.595*
	(0.509 NS)	(0.382 NS)	0.833**	0.683*
Fruiting	0.721**	0.824**	0.775**	0.662*
	(0.518 NS)	0.679*	0.601*	0.833**

only rainfall was significantly correlated with flowering in 2013–2015 (Table 1). The flowers are yellowish-green and produced in umbels (Image 1a & b); the fruit is a woody capsule (Image 1e & f).

Fruiting

The fruiting phase extended over the monsoon period (April–September) with a peak during May (Fig. 3D &

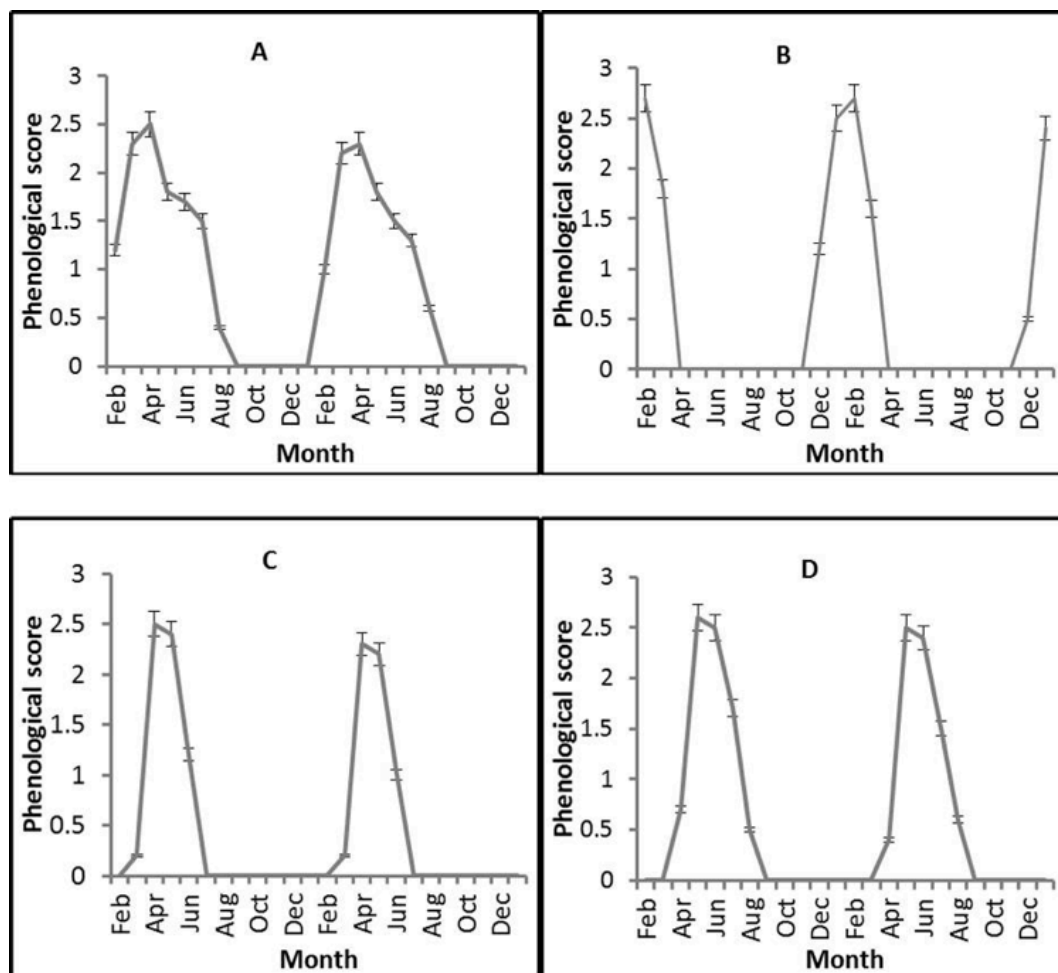


Figure 3. Periodicity of different phenophases in *A. malaccensis* during the study period (A) Leaf initiation, (B) Leaf-fall, (C) Flowering, and (D) Fruiting. Error bar represents standard error.

4). The degree of circular dispersion or concentration ($r=0.98$) indicates that the phenophase fruiting was concentrated in a particular period of the year (Fig. 5). The fruiting phenophase duration was intermediate (Rayleigh Z, $p < 0.01$), and the unripe fruits lasted for two months. The duration of fruiting phenophases ranged from 28 to 65 days with an average duration of 46.57 ± 5.24 days during 2013–14 and 30–72 days with an average duration of 51 ± 5.26 days during 2014–15, and fruiting duration varied greatly among the individuals with a coefficient of variation (C.V. % = 23.37). Fruiting presented the correlation distinctly with temperature and rainfall while in one-month lag only rainfall was significantly related with fruiting (Table 1). Availability of seasonal water had a strong impact on fruiting indicating that there was a significant relationship between one-month lag rainfall and fruiting. Fruits mature by the end of July. The fruit is a single seed which remains hanging through a small thread-like structure (Image 1f) for a few

days before dehiscence. Each seed bears a conspicuous crimson red, fleshy caruncle at the tip.

DISCUSSION

The phenological observations on the species and climatic characteristics of the study site suggest that the *A. malaccensis* is a seasonal flowering and fruiting tree species. Correlation of phenological characteristics with naturally occurring climatic events may be best documented by the pattern of leaf-fall. The greatest tendency of leaf-fall practice coincides with the relatively dry season during January–February. The timing of leaf-shedding is strongly correlated with a gradual increase in day-length, temperature, and solar insolation. This finding is in conformity with Mishra et al. (2006) who stated that maximum leaf-fall occurs during the dry period in tropical forest trees. Further, leaf-fall during this

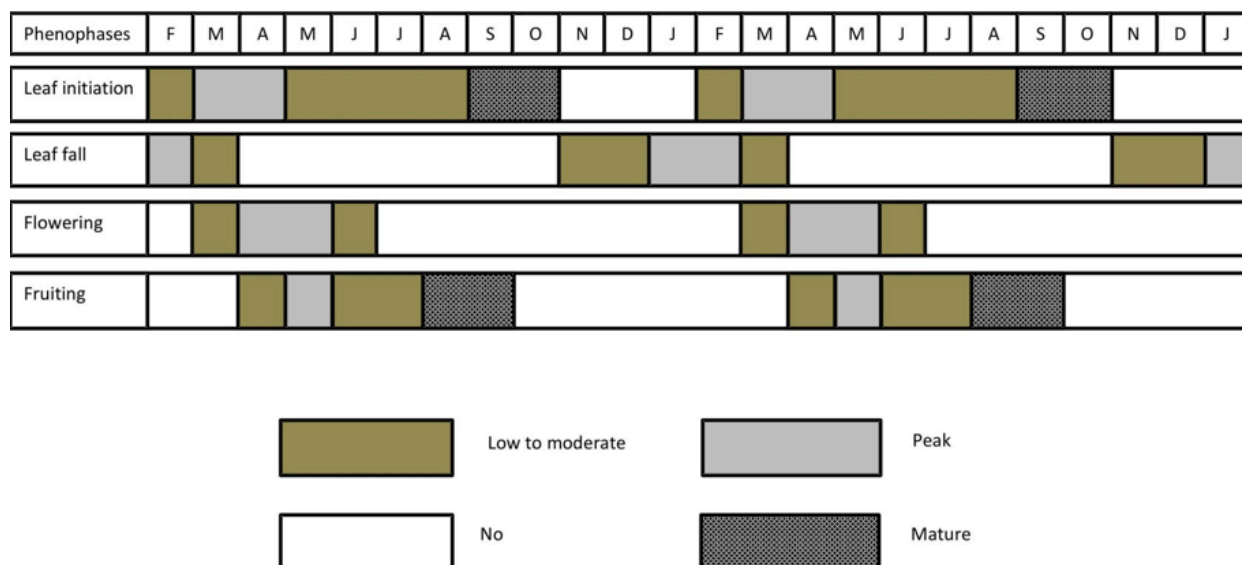


Figure 4. Vegetative and reproductive phenological events and duration recorded in *A. malaccensis* in a secondary tropical forest of Cachar District, Assam *.

* Month F=February, M=March, A=April, M=May, J=June, J=July, A=August, S=September, O=October, N=November, D=December, J=January

period appears to be an inherent strategy to minimize water loss and maximize photosynthetic activity during monsoon season (Rivera et al. 2002; Hamann 2004).

Leaf production and flushing of *A. malaccensis* start towards the end of the dry season. Short dry period, maximum temperature, and increased day length triggered the emergence of new leaves during the pre-monsoon period. The advantages of peak leaf initiation during pre-monsoon period could possibly be explained by the fact that it was to take advantage of the long rainfall period by the fully expanded foliage on trees (Singh & Kushwaha 2005). Maximum temperature and photoperiod as driving factors for leaf initiation have been reported for other tropical trees (Rivera et al. 2002; Singh & Kushwaha 2005). Saikia & Khan (2012b) observed that leaf flushing in *A. malaccensis* in home gardens starts in March and continues up to October. Species that produce leaf during the rainy season tend to have shorter periods of leaf production because this period of abundant water will normally last only for a few months. The species that greatly depend on rainfall for initiation of the leaf would also be expected to show rapid leaf growth in order to maximize photosynthetic activity during the rainy season (Reich 1995), and this type of behavior is quite common in plants growing in seasonally dry environments (Wright et al. 2002). Leaf initiation in the early rainy season is attributed to the end of the long dry season and also due to the joint action of increasing day length and temperature (Kushwaha et al. 2010). Rivera et al. (2002) have implicated that

increasing day length acts as the inducer of flushing which is relevant to the leaf phenology in *A. malaccensis*.

Flowering during the pre-monsoon season can be viewed as a strategy to make flowers more visible to pollinators and supply food sources during the poor periods of floral resources (Murali & Sukumar 1993). Species flowering during the pre-monsoon period can be capable of storing water in sufficient quantities to permit flowering even in the absence of rainfall (Borchert 1994). *A. malaccensis* as a dry season bloomer showed a significant positive correlation with photoperiod as observed for trees in tropical dry forests by Borchert et al. (2004). Soehartono & Newton (2001) reported flowering and fruiting in *A. malaccensis* growing in botanical gardens of Indonesia from April–September. Beniwal (1989) found flowering in March and fruiting in the middle of June in plantations of Arunachal Pradesh, northeastern India. Saikia & Khan (2012b) observed initiation of flowering from mid-February to May following fruiting from May and ending in August.

A. malaccensis concentrated peak fruiting during the wet season, producing dry fruits with small seeds. In tropical forests, fruiting during the rainy season may have evolved to ensure dispersal of seeds when soil water status is favourable for seed germination, seedling growth, and survival (Kushwaha et al. 2011). The requirement of moisture level for the proper development of fruits indicates that the decrease of soil water status reduced the rate of enlargement and final size of these fruits. During the wet season, availability

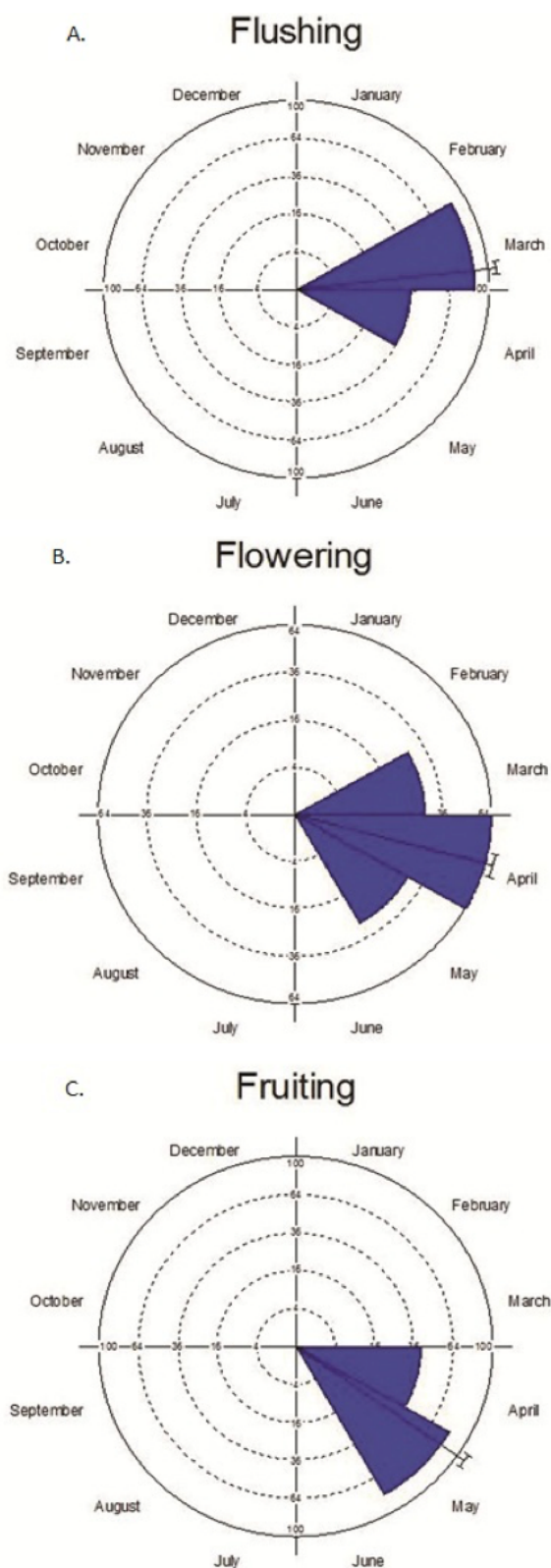


Figure 5. Circular histograms indicating the mean month of the phenophases of *A. malaccensis* in a secondary tropical forest of Cachar District, Assam. (A) Leaf flush (2013-2015: $r = 0.99$, $SD = 7.4^\circ$), (B) flowering (2013-2015: $r = 0.97$, $SD = 12.2^\circ$) and (C) fruiting (2013-2015: $r = 0.98$, $SD = 11.5^\circ$), where r = degree of dispersion or concentration and SD = circular standard deviation

of high moisture level also favours germination and establishment of seeds. The flowering phenology observed in *A. malaccensis* is reported in *Psidium guajava* and *Vatica lanceaefolia* growing in the home gardens of Barak Valley, northeastern India (Das & Das 2013). Synchronization of flowering during a particular season appears to be under the control of the prevailing climatic condition of that season (Singh & Kushwaha 2005). Maximum flowering activity during the pre-monsoon period may be related to the high insect population as pollen vectors in tropical forests. Further, seasonal flowering strategy observed in *A. malaccensis* may be a strategy to escape from seed predation on a timely basis.

In *A. malaccensis*, fruiting initiation during the rainy season is indicative of a close relationship between rainfall and fruiting, as the rainfall factor acts as a cue for reproductive phenology, especially in dry tropical forests as stated by Griz & Machado (2001). Further, autochorous seed dispersal in this species is also probably related to the humidity factor as it has an influence on fruit dehiscence. Fruit dehiscence during the monsoon season may enable the plant to escape from seed predators and produce seedlings for continued survival (Hamann 2004). Fruiting during the rainy season in tropical forests evolved to ensure dispersal of seeds and this could be attributed to utilization of available soil water for seed germination and seedling establishment (Singh & Kushwaha 2006). Tropical trees have adopted a systematic strategy so that there is adequate development time from flowering to seed dispersal so that seeds are released during the rainy period (Stevenson et al. 2008) when germination is most likely to be induced and seedlings start growing with a low probability of drought.

In the present study, the duration of flowering was longer (59.52 ± 6.36 days) and fruiting was shorter (48.78 ± 5.25 days) in *A. malaccensis* during the two years of study. This longer duration of flowering can be viewed as a difference in time taken for the formation to the maturation of buds. The short duration of fruiting is advantageous for the plant to mature fruits during the rainy season due to the availability of highest precipitation. This flowering and fruiting duration does not agree with the reports on the same in the tropical montane evergreen forest of southern India (Mohandass et al. 2016). Further, the duration of these two phenophases appears to be influenced by the changes in day length, temperature, sunshine hours, and precipitation associated with the season (Bawa et al. 2003).

The present study indicates that the vegetative and reproductive phenological events in *A. malaccensis* display a general annual flowering and fruiting pattern with a peak in these events during the pre-monsoon and monsoon seasons. Temperature and precipitation (by themselves) do not show any influence on leaf initiation but cumulatively show influence on leaf initiation. Availability of seasonal water had a strong impact on fruiting indicating that there is a significant relationship between one-month lag rainfall and fruiting. It seems that changes in temperature and rainfall pattern have a pronounced effect on the phenology of *A. malaccensis*. This information may be used as a baseline for further evaluation of phenological variations for this vulnerable tree with reference to climate change. The study suggests that there is a need to develop a long-term monitoring strategy on phenological aspects of *A. malaccensis* in order to understand the impact of climate change on phenology.

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