Annual variation in floral phenology and pollen production in *Lagerstroemia speciosa*: an entomophilous tropical tree

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Abstract

Flowering phenology and variation in total pollen production per tree in the natural population of *Lagerstroemia speciosa* was studied during four successive years, from 2006 to 2009. Reproductive phenophases were highly variable between years and individuals within population. Total flowering period lasts up to three months. Time of maximum anthesis was between 06:00 and 10:00 h of the day. On an average a flower took 2.5 to 3.0 hours for complete opening. Anther dehiscence occurred half an hour after anthesis. *Apis* spp. and *Xylocopa* spp. were observed the main pollinators. The visitation rates of pollinators varied from 0.62 to 2.82 visits/flower/hour with the average of 1.68±0.94 visits/flower/hour. *Xylocopa* bee shows physical fitness with flower morphology, which implies the functional relationship with regard to pollen transfer. There was alternate year production of flowers and pollen grains per tree. In the mass production years, a tree produces 6.4 x 10^8 and 1.3 x 10^9 pollen grains, which in poor production years oscillated between 4.2 x 10^7 and 5.4 x 10^7. 64% to 80% fruit set following open pollination in mass production year was estimated.

Keywords: anthesis, pollination, pollen production, pollinators, floral display

1. Introduction

The genus *Lagerstroemia* belongs to family Lythraceae and is known as crape myrtles, consisting of around 56 living species that occurs naturally from India, Southeast Asia, southern China, Japan and Korea to northern Australia and New Guinea (Furtado and Srisuko, 1969; Graham, 2007). *Lagerstroemia* prefers a subtropical or warm-temperate climate (Graham, 2007) and cannot survive cold winters (Qin et al., 2007). *Lagerstroemia speciosa* is a large deciduous tree that attains a height of maximum 20 m and diameter of 60 cm. It is known as Queen’s Crape-Myrtle due to the large handsome mauve flowers. The species grows naturally in northeast and southern India, throughout Myanmar, Philippines and Chittagong hills of Bangladesh (Troup, 1921). The wood is used for construction, boat-building, carts and other timber purposes and the leaves are used as medicinal purposes, e.g. controlling sugar and weight (Suzuki et al., 1999), antidiabetic (Mishra et al., 1990), and antiboest (Suzuki et al., 1999). The pollen grains of *L. speciosa* have allergic significance (Banik and Chanda, 1992; Singh and Devi, 1992). The investigation of pollen production per individual tree along with flowering phenology would be useful to the (i) aerobiologists for predicting the pollen season (starting dates of allergy) and amount of pollen emission to the air, (ii) tree breeders for making the crosses timely, (iii) foresters and silviculturists for improvement of the wood quality through provenance testing and mating design, and (iv) ecologists for natural selection and evolution of plant life history.

In biotic pollination, a large number of pollen grains are required to entomophilous species to ensure successful pollination, as the high pollen production is desirable for bee pollinated plants (Thorpe, 2000). Therefore, the pollination
success depends on pollen production. The pollen production capacity by an individual tree is under genetic and physiological control. Climatic conditions partly influence the time of anthesis, anther dehiscence and density of flower that can ultimately affect the quantity of pollen produced by an individual (Stanley and Linskens, 1974). The pollen production of an individual tree is the function of the number of pollen grains per anther, number of anthers per flower, number of flowers per inflorescence, number of inflorescences per tree, and size of individual. This data facilitate the ability of pollen emission in the air by each individuals of a species from the aerobiological view point and also valuable for forestry and agronomical sectors, because the seed production often depends on pollen production and its emission (Campbell and Halama, 1993).

Phenological observations has the ecological significance that it constitutes a dynamic approach for evaluation of plant reaction to the local environment and also play a key role to provide knowledge on functional rhythms of plant and plant communities (Poole and Rathcke, 1979). Adaptation of the phenological events provide platform for the utilization of species resources that lead temporal periodicity and temporal separation of species (Connel and Orias, 1964). Knowledge of floral display, anthesis and pollen production along with phenological observations is important to the study of pollination and functional model development for forecasting pollen concentrations. Therefore, this study of temporal variation in phenology and pollen production in L. speciosa shall be of great value for theoretical model-building as well as for the development of resource management concept, which is widely applicable in plant breeding and tree improvement, silviculture, assessment of plant growth rate and the prediction of evolutionary changes.

2. Materials and Methods

2.1 Study site

The study was conducted during four successive years in each annual flowering season from 2006 to 2009 of Lagerstroemia speciosa growing naturally around Luangmual area on the way to Mizoram University campus from Aizawl City. The average annual temperature of the area is 24.7°C and the average total precipitation for the study years (4-years) is 1,872 mm, with the main rainy season between May and September (Figure 1). The forest has been used for logging and lopping and has a major impact on vegetation or on the abundance and distribution of animals. Five trees were selected randomly for studying each trait, i.e. floral phenology, pollinators, pollen production and pollination. Same trees were used every year for observations.

2.2 Floral phenology and floral display

Phenological observations on selected individuals were made in terms of timing of leaf initiation, timing of floral bud initiation, timing of first flowering, duration of flowering, end of flowering, fruit maturity, and seed dispersal by visual observation on fortnightly basis during vegetative phase and weekly basis during reproductive phase. The timing of all the above mentioned parameters was different among the selected individuals, therefore, the dates of each variable were recorded separately on every selected individuals and the average dates for every parameter has been summarized in Table 1.

2.3 Flower visitors and pollination

Determination of pollinators and their visitation rates was done by counting the visiting insect species on all the chosen trees. The study period covered the 1.5 month flowering season (mid-May to June). Several inflorescences were observed simultaneously and the number of insects visited to

<table>
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<th>Observed variables</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timing of leaf flushing</td>
<td>8th March</td>
<td>2nd April</td>
<td>24th March</td>
<td>9th March</td>
</tr>
<tr>
<td>Timing of floral bud initiation</td>
<td>6th April</td>
<td>20th April</td>
<td>15th April</td>
<td>10th April</td>
</tr>
<tr>
<td>Timing of start of blooming</td>
<td>28th April</td>
<td>14th May</td>
<td>10th May</td>
<td>29th April</td>
</tr>
<tr>
<td>Period of blooming</td>
<td>Up to 30th June</td>
<td>Up to 20th June</td>
<td>Up to 26th June</td>
<td>28th May</td>
</tr>
<tr>
<td>Timing of end of flowering</td>
<td>10th July</td>
<td>2nd July</td>
<td>5th July</td>
<td>Up to 19th June</td>
</tr>
<tr>
<td>Timing of seed dispersal</td>
<td>April 2007</td>
<td>Mid-March 2008</td>
<td>April 2009</td>
<td>Mid-March 2010</td>
</tr>
</tbody>
</table>

Figure 1. Variation in total monthly rainfall during study years.
the open flower was recorded. Each tree was observed over the course of the whole day between 6:00 and 18:00 in 12 observation blocks each starting at every full hour, i.e. between 06:00–07:00, 07:00–08:00, 08:00–09:00, etc. The observation blocks were randomly distributed over the trees and over the course of the day. The frequency of pollinators was assessed in terms of visits/flower/hour.

2.4 Flowers and pollen production

Production of flowers and pollen grains per tree was also recorded on the selected trees. In *L. speciosa* the inflorescences are large and easily countable on the crown of the 6 to 8 m high tree. At first, all the inflorescences on the crown of a selected tree and subsequently for five trees was counted manually, then five inflorescences per tree were chosen randomly throughout the crown and harvested to count the number of flowers per inflorescence. From the harvested inflorescences, twenty five flowers (five from each inflorescence) were taken and the numbers of anthers per flower were counted manually. Furthermore ten anthers from different flowers were chosen and the numbers of pollen grains per anther were counted by crushing the anther in a microscopic slides containing one drop of 50% glycerol. The pollen grains were counted under a binocular microscope. Pollen production per tree was estimated by following equation:

\[
\text{Total pollen grains/tree} = \frac{\text{Number of inflorescences/tree} \times \text{average number of flowers/inflorescence} \times \text{average number of anthers/flower} \times \text{average number of pollen grains/anther}}{600 \text{ per h}}
\]

2.5 Fruit set

Fruit setting from open-pollination was also recorded on five selected individuals by choosing five inflorescences in each tree that were flagged. The observations were made in December after five to six months of pollination by counting the number of fruits produced by an inflorescence and subsequently by five inflorescences in each tree. Fruit setting was calculated by dividing the average number of fruits per inflorescence by the average number of flowers per inflorescence at pollination.

3. Results

3.1 Floral phenology and floral display

The vegetative and reproductive phenophases were highly variable from one year to the next and also from one individual to the next in a population. There was 5 and 14 days difference in the timing of floral bud initiation from one year to the next. The blooming period was also varied greatly from year to year. The timing of different reproductive phenophases over four different years has been displayed in Table 1. This fluctuation in reproductive phenology could be due to precipitation level in the preceding year. The monthly precipitation during four years has been presented in Figure 1. Further the observations revealed that the flowering period of *L. speciosa* lasts up to three months. Flowering was simultaneous in an inflorescence from basal portion to apical portion of the inflorescence, which took one month for complete blooming. However, there was an overlap in flowering among individuals in a population. Fruit setting and development of fruits takes place one week after pollination. Anthesis and fruit development occurred simultaneously in an inflorescence. The detailed display of the flower is given in Table 2. Maximum anthesis was observed during morning hours between 06:00 to 10:00 h of the day. A flower took 2.5 to 3.0 hours for complete blooming. Anther dehiscence occurred half an hour after anthesis.

Between years the differences in all reproductive parameters were observed, which appear to be ample variation in reproductive output between individual plants in a population. Between individuals in any one year, there was variation in the timing of first flowering, flowering peak, and duration of flowering (Table 1). For each component of flowering phenology, the amount of variation varied considerably between years as there was a broad spread of first flowering dates in 2006 and 2009 than in 2007 and 2008. For an individual tree, timing of first flowering and duration of flowering was consistently followed between years. Thus, early flowering individuals in one year also flowered early in the next year and plants with long flowering periods in one year had long flowering periods in the next. Timing of peak flowering and overlap was not consistent between years; individual plants varied from year to year in the exact timing of their peak flowering period and flowering overlap with the rest of the population.

3.2 Pollinators and their frequency

The main pollinators observed in *L. speciosa* were honey bees (*Apis* spp) and carpenter bees (*Xylocopa* spp). The interaction of both pollinators with the reproductive parts of flowers is shown in Figure 2. The maximum frequency of the pollinators was recorded between 07:00 to 09:00 h of the day. The number of pollinators per square meter was 25±2.14 (honey bees) and 10±0.98 (Carpenter bees). The frequency of pollinator’s visitation per inflorescence per hour was 52±12.46. However, visitation rates differed among individuals in the population which varied from 0.62 to 2.82 visits/flower/hour and the average visitation rates per flower was 1.68±0.94 visits/flower/hour. It is interesting to point out that at 07:00 h first three to four honey bees visited the flower; then they went back and brought groups of bees after half and hour. This was not observed in case of carpenter bees. The receptivity of stigmas lasts up to two days and the receptive stigmas were green in color, which could be used as identification marker for judging the receptivity in *L. speciosa*. 

3.3 Flowers and pollen production

In *L. speciosa*, production of the number of inflorescences per tree varied considerably from one year to the next. In the mass production years the value ranged between 76 and 180 (2006) and 88 and 172 (2009) inflorescences per tree whereas in poor production years the value oscillated between 8 and 15 (2007) and 10 and 15 (2009). The variation was eleven folded between mass and poor production years. The flowers per inflorescence were also higher in mass production years. However, the anthers per flower and pollen grains per anther did not vary between mass and poor production years (Table 3). Average pollen grains per tree in *L. speciosa* during mass production years were 6.4×10⁸ to 1.3×10⁹ in 2006 and 7.4×10⁸ to 1.04×10⁹ in 2008. Whereas in poor production years the values were recorded as 4.2×10⁷ to 5.2×10⁷ in 2007 and 5.0×10⁷ to 5.4×10⁷ in 2009 (Table 3). Mass production years proclaimed higher fruit setting percentage as compared to poor production years following open pollination. The capsule like fruits contains 5 to 6 valved, broadly...
Table 3. Flower and Pollen production per tree in *L. speciosa*.

<table>
<thead>
<tr>
<th>Year and Tree No.</th>
<th>Height (m)</th>
<th>Diameter (cm)</th>
<th>Inflorescences/tree</th>
<th>Flowers/Inflorescence</th>
<th>Anthers/Flower</th>
<th>Pollen grains/Anther</th>
<th>Pollen grains/Flower</th>
<th>Pollen grains/Inflorescence</th>
<th>Pollen grains/Tree</th>
<th>Fruits/Inflorescence</th>
<th>Fruit setting (%) (open pollination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
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<tr>
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<td>8</td>
<td>28</td>
<td>180</td>
<td>32±4.16</td>
<td>265±25.18</td>
<td>870±102.10</td>
<td>230550</td>
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<tr>
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<td>21</td>
<td>130</td>
<td>37±6.10</td>
<td>248±20.14</td>
<td>920±116.96</td>
<td>228160</td>
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<td>6</td>
<td>18</td>
<td>104</td>
<td>26±9.12</td>
<td>290±30.46</td>
<td>816±130.62</td>
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ovoid with the average length and circumference of 4.6±0.46 and 7.4±0.80 cm respectively. The seeds are light brown, angular fairly hard, with a stiff brittle wing. On an average per capsule contains 152±14.18 seeds.

4. Discussion and Conclusion

4.1 Floral phenology and floral display

Almost all the vegetative shoots entered to the flowering phase and produced many flowers in the mass flowering years. The blooming and fruiting period was relatively long. The fruit maturation and seed dispersion occurs in winter and spring simultaneously taking almost 6 and 9 months from pollination, which coincides with the second years’ active growth phase resulting in the failure of reproductive growth to the next season. Contrary to this, the individuals that have been lopped by farmers for their livelihood sustenance produced reproductive growth every year. The long blooming period along with the high flower production are life-history traits that have been associated with plants of disturbed habitats (Grime, 1979).

The average length and width of petals was 5.2±0.86 and 4.8±0.64 cm, respectively, which results in a large diameter (10.6±1.26) of the flower. Large flowers may have higher pollinator visitation, pollen production, pollen removal, fertilization success hence sire more seeds than small flowers (Harder and Barrett, 1995). Morphological fitness of Xylocopa bee to the flower of L. speciosa was observed (as shown in Figure 2B), which implies the functional relationship between pollinators and morphologies of flower with regards to pollen transfer (Wilson et al., 2004). Therefore, the shape and size of the flower can influence reproductive output by affecting the behavior of pollinator and rates of visitation (Galen and Newport, 1987).

The flowering phenological traits, e.g. onset, duration and synchrony of flowering among individuals in a population revealed a high degree of variation. However, commendable overlapping in flowering within the population was also apparent that has been considered as the population level flowering synchrony in L. speciosa. The population level flowering synchrony in L. speciosa has the potential benefit to increase pollinator attraction (Augspurger, 1981) and outcrossing frequency (Rathcke and Lacey, 1985). Nevertheless, flowering synchrony also has strong breeding consequences in the population. The higher the synchrony within a population, the fewer the number of individuals a plant can potentially exchange genes with in a given reproductive period(Primack, 1980).

4.2 Pollinators and their frequency

The daily pattern of pollinators visitation was related to the pattern of anthesis of the flowers. No nocturnal visitors were observed. Considerable day to day variation in pollinators visitation was observed, which is supported by many other studies (Herrera et al., 2001; Lazero and Traveset, 2005; Brunet and Sweet, 2006). The pollinators, especially honey bees and carpenter bees, feed on pollen in addition to nectar. The amount of pollen was highest in the morning, as the anther dehiscence took place half an hour after anthesis, leading to high visitation rates at that time. Similar pattern was recorded for Neodypsis decaryi, a Malagasy palm (Ratsirarson and Silander, 1996). The recorded visitation rate of L. speciosa is higher than that reported for Commophora guillaumini (Farwig et al., 2004) and comparable to those recorded for several other species (McCay and Primack, 1992, Ratsirarson and Silander, 1996 Ghazoul, 1997). The results of floral biology and pattern of pollinator visitation to the flowers of L. speciosa may cause sporadic outcrossing and mixed mating in a population despite there is a synchrony in flowering among individuals.

4.3 Production of flowers and pollen grains

Annual production of flowers and pollen grains varied considerably from one year to the next (Figure 3), with almost total failure of flowering between two production years. The inter-annual production of flower may be due to morphological, physiological, and general mass flowering phenomena that occur at irregular intervals. Mass flowering is a notable feature of many seasonal forests in Southeast Asia. This is called supra annual pattern of flowering, and it may involve one species, a group of related species, or a majority of species in a community. Yap and Chan (1990) recorded three mass flowering years in 1976, 1981, and 1983 over a period of 11 years, while observing 310 trees belonging to 16 species of Shorea at four sites. Khanduri and Sharma (2009) also reported mass flowering in Cedrus deodara after every alternate years with total crop failure in 50% individuals between two mass production years in a population over a period of seven years. Their study showed that the mass flowering is regulated by the precipitation level. The mass flowering is generally linked with environmental cues, i.e. prolonged drought (Janzen, 1974). Nevertheless, mass flowering in L. speciosa was not linked with the level of precipitation;
therefore it was not triggered by climatic factors but was due to morphological factors, i.e. the previous years fruits bearing inflorescences are situated in such a position that no floral bud can be formed at that place in the following year. This was verified by observing the trees which were lopped by the farmers annually during winter in all the four observation years. The lopped trees flowered annually with almost equal intensity, though; the lopped trees were not sampled but visually observed every year.

The production of pollen grains per tree in *L. speciosa* revealed a partial estimate of an individual pollen emission and accumulates new knowledge on the contribution to the airborne pollen content from a particular species. If the density of the individuals in the population is known, approximate estimation of total pollen release to the atmosphere to cause pollinosis could be made. Nevertheless, the quantity of pollen emission depends on the duration of flowering period and also on the meteorological factors which would influence the seasonal pollen fluctuations.

In conclusion, the results of floral biology and pattern of pollinator visitation to the flowers of *L. speciosa* may cause sporadic outcrossing and mixed mating in a population. Further, the production of total flower and pollen grains per tree with the interaction of pollinators and flowering phenology influence the genetic composition of the population.

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