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Contribution of Diurnal and Nocturnal Insects to the Pollination of *Jatropha curcas* (Euphorbiaceae) in Southwestern China

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ABSTRACT *Jatropha curcas* L. (Euphorbiaceae) is being increasingly planted worldwide, but questions remain regarding its pollination biology. This study examined the contribution of diurnal and nocturnal insects to the pollination of monoecious *J. curcas*, through its floral biology, pollination ecology, and foraging behavior of potential pollinators. Nectar production of both male and female flowers peaked in the morning, declined in the afternoon, and rapidly bottomed during the night in all of their anthesis days. The diurnal visitors to the flowers of *J. curcas* are bees and flies, and the nocturnal visitors are moths. Flowers received significantly more visits by diurnal insects than by nocturnal insects. Through bagging flowers during night or day or both or exclusion, we compared fruit and seed production caused by diurnal and nocturnal pollinators. Both nocturnal and diurnal visitors were successful pollinators. However, flowers exposed only to nocturnal visitors produced less fruits than those exposed only to diurnal visitors. Thus, diurnal pollinators contribute more to seed production by *J. curcas* at the study site.

KEY WORDS *Jatropha curcas*, diurnal pollination, nocturnal pollination, female reproductive success, nectar production

Jatropha curcas L. (Euphorbiaceae) is a tree thought to be native to Central America (Fairless 2007) and possibly Brazil but now is grown widely in other parts of the world, including China (Sun et al. 2008). It is estimated that there are already 500,000–600,000 ha of *J. curcas* planted in India, and 2 million ha under cultivation in China (Fairless 2007). The wide adoption of this plant is mainly due to a growing interest in the use of *J. curcas* seed oil to meet the ever-increasing demand for renewable energy sources. *J. curcas* seed oil is relatively simple to convert into biodiesel through either chemical (Berchmans and Hirata 2008) or biological transesterification (Modi et al. 2007). *J. curcas* biofuel is not only inexpensive to produce but also is reported to be nontoxic, clean, and ecofriendly (Jha et al. 2007). To maintain a high yield of seed production for nonwind-pollinated plants, we must first understand the role of pollinators in sustaining pollination for each plant species.

J. curcas exhibits diurnal floral traits associated with short-proboscis pollinators, such as bees or flies; however, flowers remain open during the night and thus also may be pollinated by nocturnal visitors. Many studies have referred to diversified diurnal pollinators

of this plant (Heller 1996, Raju and Ezradanam 2002, Bhattacharya et al. 2005, Luo et al. 2008). These studies examined the role of insect pollination relative to that of wind pollination or apomixes. However, they did not examine the contribution of nocturnal insects to seed set. Similar diurnal observations on flower visitors were made for three other *Jatropha* species: *Jatropha mutabilis* (Pohl) Baill, *Jatropha mollissima* (Pohl) Baill (Santos et al. 2005), and *Jatropha gossypifolia* L. (Reddi and Reddi 1983). Dehgan and Webster (1979) proposed that moths might act as nocturnal pollinators to *J. curcas* because its flowers are greenish white with inconspicuous nectar guide and produce sweet, heavy perfume at night. If moths act as nocturnal pollinators, what role do they play in the female reproductive success of the plant? What is the pollination strategy of *J. curcas* in a hot arid valley where the local abundance of pollinators varies temporally and spatially?

In this study, we examined: 1) floral characteristics and nectar production by *J. curcas*; and 2) the contribution of diurnal and nocturnal flower visitors to seed set in *J. curcas*.

Materials and Methods

Study Site. This study was conducted in *J. curcas* stands near Shuangbai in the hot, arid Honghe Valley, Yunnan, southwestern China. The area is located at 24° 22.447' N, 101° 39.164' E. Elevation is ≈647 m above sea level. Annual average temperature and precipitation is 23.4°C and 523.1 mm, respectively, and 86.5% of the

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precipitation occurs during the rainy season (from late May to late October). Climatic data were acquired from HL20 System Monitor (Jauntering International Corporation, Taiwan, China) installed near the *Jatropha* stand. The vegetation in the area of the site was open savanna grassland, but herbaceous plants included *Heteropogonetea contortus* (L.) P. Beauv ex Roem & Schult and the woody species *Eriolaena wallichii* Candolle and *Pachystachys lutea* Nees. No insect control was conducted in study area for area of farmlands is very small (≈ 1 ha), and no major crops were planted there.

Floral Biology. *J. curcas* is a perennial deciduous shrub or treelet. In the hot, arid valley of southwestern China, it flowers from the dry season to the rainy season with flowering concentrated from mid-April to late May before the rainy season starts. The flower is unisexual, and male and female flowers are produced in the same inflorescence at the branch terminals. Each inflorescence consists of from zero to 17 female flowers from 49 to 238 male flowers (Luo et al. 2007). Both flower sexes open at early morning (0800–0900 hours); female flowers usually bloom for 5 d, whereas most male flowers wither by dusk of the second day (Luo et al. 2007).

Previous studies have reported a few floral characteristics of *J. curcas*, including floral structure, color, and scent type (Raju and Ezradanam 2002, Luo et al. 2007). We selected six flowers in each plant (30 male and 30 female open flowers in total) in May 2009 and preserved in formalin:acetic acid:ethanol (70%, 1:1:18, vol/vol/vol) for further examination. We recorded the following features of floral parts: 1) corolla diameter, 2) diameter of pistil and stamen, 3) length of floral tube and sexual organs, and 4) emission time of floral fragrance. Animal-pollinated plants influence their mating success through characteristics of their individual flowers (Ishii and Harder 2006).

Nectar Production. To characterize the nectar secretion pattern, we selected unopen male ($N = 20$) and female flowers ($N = 22$) from plants (one flower per plant) and protected it with pollinator-excluding bridal veil from floral visitors in May 2009 (Ibarra-Cerdena et al. 2005). Nectar was repeatedly collected with 0.4- μ l capillary tubes from all five nectaries of each flower, and nectar length was measured with a vernier caliper accurate to 0.02 mm, separately at dawn (0730 hours), early afternoon (1330 hours), and dusk (1830 hours) each day. This schedule was chosen because we sampled some flowers for an entire day and found that the amount of nectar production was too small to be collected frequently (usually < 0.1 μ l/d). We then calculated nectar volume and secretion rate by dividing nectar volume by the nectar accumulation time. We were careful not to damage the ovary (or stamen) of flowers, which might affect floral longevity. For male flowers, the experiment was continued for two consecutive sunny days, after which most male flowers started wilting and falling off, indicating the end of male flower development. The nectar production of female flowers was measured for

the first three consecutive days when the stigma receptivity is high.

Pollinator Assemblage and Activity. In parallel with the observations of floral biology, we also made periodic observations of the fauna associated with ten randomly selected inflorescences (one per plant). Observations were made every 3 h of each sunny day. In each observation, we counted all pollen-collecting or nectar-feeding insects directly on the inflorescence. The daily sampling effort was, on average, 24 min per inflorescence (≈ 3 min per inflorescence per observation). The observations were made for 6 d. Therefore, the total sampling effort added up to 24 h per 10 inflorescences (24 min \times 10 inflorescences \times 6 d).

Diversity, abundance, and activity of diurnal and nocturnal visitors were observed as follows. For each insect visiting an inflorescence, we recorded its taxonomic group, time and duration of visit, and whether legitimate (i.e., visitor's body contacted anthers, stigma, or both), or illegitimate (no contact with anthers or stigma). For nocturnal observations, a flashlight covered with a red filter was used. We made a preliminary investigation of nocturnal visitors to the flowers of *J. curcas* in 2008 and found moths visiting the flowers. To identify diurnal and nocturnal visitors and estimate their pollen transfer capability, we captured visitors once a week with an insect net in 2009, for 2 wk during the *J. curcas* flowering season, after the pollinator effectiveness observation had been completed. We tried not to contact the abdomens and proboscises of the visitors, which could potentially carry pollen. We checked the visitors' abdomens and proboscises for *J. curcas* pollen grains (by comparing a known sample of *J. curcas* pollen) under a microscope. To evaluate the effectiveness of honey bees as pollinators, we exposed virgin stigmas to a single visit by honey bees, and then collected them. We determined the number of pollen grains deposited under a dissecting microscope because pollen grains are large (≈ 95 μ m in diameter, Bhattacharya et al. 2005), not too numerous, and the stigmatic surface is smooth.

Pollinator species were organized into functional groups, based on their similar behaviors on the flowers (Armbruster et al. 1999, Fenster et al. 2004), as honey bees, solitary bees, flies, beetles, and moths. Voucher specimens were deposited at entomology museum of Research Institute of Resource Insect, Chinese Academy of Forestry. Ants also were found visiting *J. curcas* flowers (Raju and Ezradanam 2002, Luo et al. 2008), but we did not include ants in the pollinator list because of their limited potential as pollinators (Janzen 1977) due to small size, smooth and hairless cuticles, and low mobility (winglessness).

Pollinator Effectiveness. To test for the presence and effectiveness of diurnal and nocturnal pollinators, we bagged entire inflorescences at different times. Four different treatments were assigned to 377 marked female flowers. Each treatment consisted of eight to 25 flowers from each plant, and every treatment was replicated six times:

Diurnal Pollination. Flowers ($N = 92$) were bagged during the night and bags were removed when diurnal

pollinators began to forage on flowers (bagged 1940–0630 hours).

Nocturnal Pollination. Flowers ($N = 100$) were left exposed to nocturnal pollinators and then bagged before diurnal pollinators were active (bagged 0630–1940 hours).

Open Pollination. Flowers ($N = 95$) were labeled and left unbagged and were thereby available to both nocturnal and diurnal pollinators.

Exclusion of Pollinators. To determine whether fruit set occurred in *J. curcas* without insect pollinators, some flowers ($N = 90$) were bagged both during the day and night.

All experiments were conducted on consecutive days at dawn (0630 hours) and dusk (1940 hours) because honey bees, solitary bees, and flies were observed foraging at 0700 hours at the earliest and 1930 hours at the latest, and moths began visiting flowers after 2000 hours. The observation started from the first female flower's opening until all the female flowers of the marked inflorescence were deemed senescent when the stigmas turned black, and no pollen adhered to it. For each of the four treatments, we recorded whether flowers aborted or produced mature capsules. Mature capsules were collected for seed counting 8 wk after the experiments. Care was taken to collect the capsules before they dehisced, thereby preventing seed loss. Seeds were counted for each capsule. We used both fruit set and seed numbers per capsule as estimates of female reproductive success.

Statistical Analyses. Floral morphological parameters were compared with Student's *t*-tests. The nectar production rate within each time intervals was analyzed with one-way analysis of variance (ANOVA). The effect of nocturnal and diurnal pollination on fruit set and seed numbers per capsule was analyzed by ANOVA and least significant difference (LSD). Seed numbers per capsule was transformed $\log(x + 1)$ to meet the requirement of normal distribution and homoscedasticity of ANOVA. The distribution of pollinators in different functional groups and different time intervals were analyzed by Kruskal–Wallis tests. All analyses were carried out with SPSS 11.5 (SPSS Inc., Chicago, IL). Means were given with their SEs throughout the text.

Results

Floral Biology. Significant differences were found in some parameters between male and female flowers, such as corolla diameters and sexual organ (pistil or stamen) diameters; other parameters were the same, such as corolla tube lengths and sexual organ lengths. Corolla diameter of female flowers was larger than that of males (8.47 ± 0.26 vs. 7.09 ± 0.38 mm for female and male flowers, respectively; *t*-test: $df = 58$, $P < 0.05$). The corolla tube length of male flowers was 4.47 ± 0.14 mm and that of females was 4.44 ± 0.16 mm, with no significant difference (*t*-test: $df = 58$, $P > 0.05$). The diameter of stamen (4.31 ± 0.12 mm) was larger than that of pistils (2.34 ± 0.17 mm) (*t*-test: $df = 58$, $P < 0.05$). The lengths of stamen and pistil were

7.58 ± 0.16 and 7.59 ± 0.10 mm, respectively (*t*-test: $df = 58$, $P > 0.05$); both are significantly longer than their corolla tubes (*t*-test: $df = 58$, $P < 0.05$). Flower of both sexes emitted slightly sweet odor after open. The scent is relatively strong at early morning and then become faint in the afternoon. The floral odor during the night, however, varied from faint to relatively strong.

Nectar Dynamics. Nectar production started early in the morning after flowers were open, and both flower sexes secreted small amounts of nectar. Daily nectar production for male flowers during the first and second days was 0.081 ± 0.014 and 0.050 ± 0.020 μ l, respectively, and the total nectar production for both days was 0.13 ± 0.03 ($N = 20$). Daily nectar production for female flowers during the first, second, and third days was 0.071 ± 0.011 , 0.068 ± 0.016 , and 0.066 ± 0.021 , respectively, with an accumulated 3-d production of 0.21 ± 0.03 ($N = 22$).

Significant differences in nectar secretion rate among the hours of collection nested within the individual plants were observed in male flowers for the first day ($F = 31.6$; $df = 2, 57$; $P < 0.001$; Fig. 1); and female flowers during the first ($F = 103.2$; $df = 2, 63$; $P < 0.001$), second ($F = 44.5$; $df = 2, 63$; $P < 0.001$), and third days ($F = 22.3$; $df = 2, 63$; $P < 0.001$; Fig. 1). We did not test for secretion differences for male flowers on the second day because we only collected two data points.

Flower Visitors. There were five functional groups of insects in total recorded foraging on the flowers of *J. curcas*. Diurnal visitors were more abundant than nocturnal visitors, and most of the visits were done by diurnal visitors (Fig. 2). Diurnal visitors included honey bees (*Apis cerana* F. and *Apis florea* F.), solitary bees (*Delta conoideum* Gmelin, *Sceliphron modraspatanum* F., and *Campsoneris phalerata* Saussure), flies (*Chrysomya megacephala* F. and *Lucilia porphyrina* Walker), and beetles (*Hypomeces squamosus* F.). All species were identified as visitors to the flowers of *J. curcas* previously (Luo et al. 2008), except *H. squamosus*. *A. cerana* and *A. florea* deposited an average of 31 ± 7 ($N = 6$) and 20 ± 16 ($N = 8$) pollen grains on the virgin stigma, respectively. The nocturnal visitors were moths (*Hypocala subsatura* Guenee, *Hypocala moorei* Butler, and *Melanitis leda* L.). Less than 10% of the visits were done by moths (Fig. 2). All these visitors had pollen grains of *J. curcas* adhered on their body hairs. For diurnal visitors, pollen grains were found on several body parts, such as the proboscis, abdomen, and legs. For moths, pollen grains were mostly on the proboscis.

Diurnal visitors started their visits early in the morning, peaking near midmorning, and tending to decrease their activity during the afternoon and evening. Nocturnal visitors appeared early in the night (Fig. 2) and kept a sustained activity until dawn. Significant differences were observed among groups [Kruskal–Wallis test: $H(4, n = 30) = 12.62$, $P < 0.05$]. Statistically significant differences were also found among hours of the day nested within group [Kruskal–Wallis test: $H(7, n = 30) = 64.09$, $P < 0.001$].

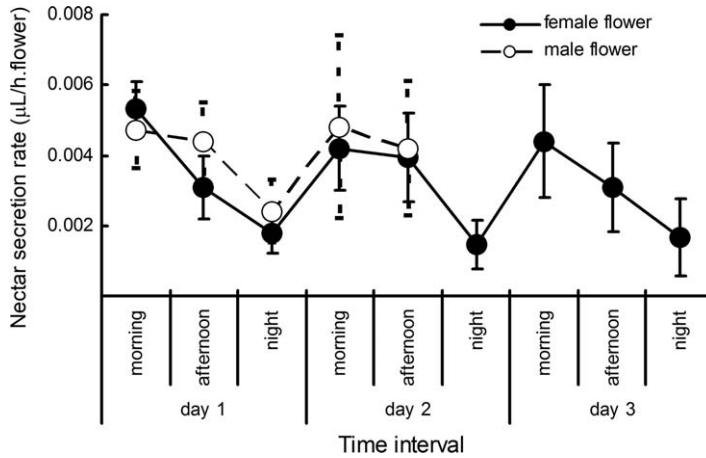


Fig. 1. Mean \pm SE nectar secretion rates in the morning, afternoon, and evening over the flowering period for male ($N = 22$) and female ($N = 20$) flowers in *J. curcas* in the arid hot Honghe Valley, southwestern China.

Pollination Effectiveness. Flowers in unbagged, diurnal, nocturnal, and excluded treatments produced ripe capsules after ≈ 60 d. Differences in fruit set among four treatments were statistically significant ($F = 63.39$, $df = 3$, $P < 0.001$). There was a significant difference in fruit set values between flowers bagged at night and those bagged during the day ($P < 0.05$; LSD test) (Fig. 3). The flowers open to diurnal pollinators did not differ significantly from the unbagged treatment in the proportion of fruits produced ($P = 0.52$; LSD test) (Fig. 3). Fruit set resulting from visitation by nocturnal pollinators was significantly more than that of flowers always bagged ($P < 0.001$; LSD test) (Fig. 3). Flowers that were bagged both day and night yielded only a few fertile capsules (0.056 ± 0.074). No significant difference was found in seed numbers per capsule among the four treatments (ANOVA: $F = 2.96$, $df = 3$, $P > 0.05$) (Fig. 3).

Discussion

Floral Characteristics and Nectar Production. *J. curcas* has diurnal, greenish white, actinomorphic, bowl-shaped small flowers with exposed sexual organs that allows easy access to nectar and pollen. These features attract a wide array of floral visitors, especially those with short mouthparts, as a broad spectrum of unspecialized pollinators, including small bees (Bawa and Opler 1975, Bawa 1994). Most honey bees and flies visiting the flowers of *J. curcas* have short proboscides varying from 2 to 5 mm (Reddi and Reddi 1983; C.W.L., personal observations), which are compatible with the length of the floral tubes of this species. Small visitors can be inefficient pollinators in many other plants due to their size. However in *J. curcas*, a small-flowered species with exposed sexual organs and a few ovules, these insects seem to be very efficient pollinators.

Nectar production between male and female flowers was unimodal with an abrupt increase and rapid

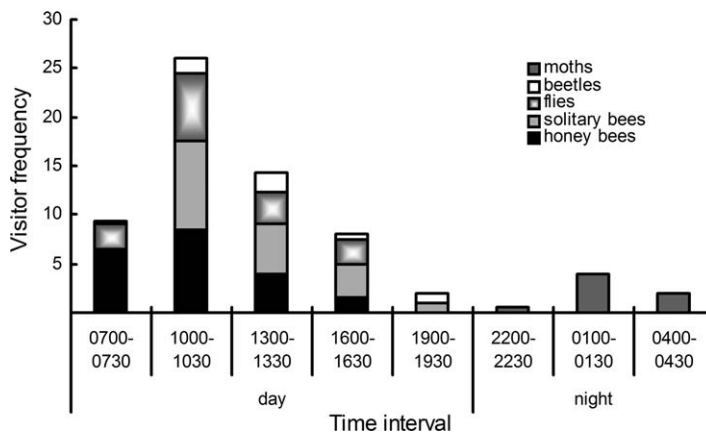


Fig. 2. Diurnal and nocturnal variation in mean frequency (number of visitor per 10 inflorescences in 30 min, 3 min per inflorescence per observation) of different groups of visitors to flowers of *J. curcas* in 6 d.

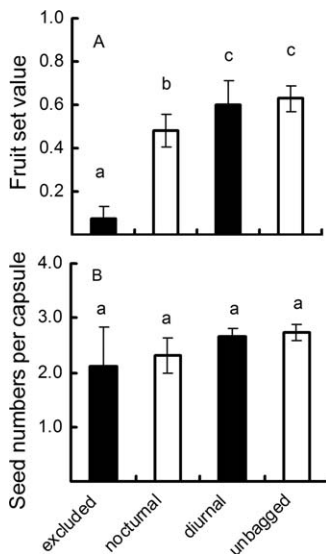


Fig. 3. Fruit set value of flowers (A) and seed numbers per capsule (B) formed from flowers subject to the four pollination treatments: excluded, nocturnal pollination, diurnal pollination, and unbagged flowers. Different letters on top of each bar indicate significant differences from one another by LSD ($P < 0.05$) after significant effect found by ANOVA.

decrease, reaching the maximum of nectar supply for diurnal pollinators around midmorning (Fig. 2). Luo et al. (2007) also found that both female and male flowers of *J. curcas* open synchronously from 0800 to 0900 hours, with a sweet aroma, high pollen viability, and stigma receptivity, and that these features decline during the day. Therefore, this species seems to have floral opening and nectar production patterns that maximize visitation by its most effective pollinators, as is often the case with other species (Witt et al. 1999, Arizaga et al. 2000, Ibarra-Cerdena et al. 2005). Similar synchronization among flowering time, peak in nectar production, and peak of visitors occur in many Euphorbiaceae species (Reddi and Reddi 1983, Steiner 1983, Vieira and Carvalho-Okano 1996). Because we did not measure nectar concentration due to small volume, we did not know whether nectar concentration in the flowers of *J. curcas* changed with nectar volume, as in *Stenocereus queretaroensis* (Weber) Buxbaum (Cactaceae) (Ibarra-Cerdena et al. 2005), or not, as in *Rhododendron ponticum* L. (Ericaceae) (Mejías et al., 2002). But studies of several other *Jatropha* species reported no significant variations in nectar concentration during their anthesis periods (Reddi and Reddi 1983, Santos et al. 2005).

Relative Effectiveness of Diurnal and Nocturnal Pollinators. Our study showed that fruit set in *J. curcas* was significantly higher in diurnal than in nocturnal treatment, with the diurnal treatment equal to the open unbagged treatment (Fig. 2). Thus diurnal pollinators were more abundant and effective than nocturnal ones at our study site. Many other studies also have found that diurnal insects are more abundant than nocturnal insects and their overall effectiveness

is greater, for other plant species (Jennersten and Morse 1991, Guitian et al. 1993, Miyake and Yahara 1998, Slauson 2000). Among a diverse range of diurnal visitors to the flowers of *J. curcas*, bees and flies are the most common, effective pollinators (Raju and Ezradanam 2002, Luo et al. 2008), whereas butterflies might be significant visitors due to their large numbers near farmland in Yuanjiang, Yunnan (Luo et al. 2008).

Nevertheless, nocturnal pollinators also contributed to a substantial proportion of fruit set, compared with flowers excluded from any pollinators. Some floral characteristics in *J. curcas*, such as greenish white color and sweet scent at night, may help guide moths to flowers and thus increase their visitation frequency. Numerous studies showed that nocturnal visitors could play an important role in female reproductive success of plants because of their higher efficiency (on a per-visit basis) (Fleming and Holland 1998, Arizaga et al. 2000, Young 2002, Ibarra-Cerdena et al. 2005). Although we did not evaluate the per-visit effectiveness in this system, we can infer that the per-visit effectiveness of the moths must be particularly high in this species, because even though overall pollinator effectiveness (fruit set) was lower for nocturnal visitors than for diurnal visitors, yet the abundance of nocturnal ones was much lower compared with diurnal ones.

Furthermore, the relative contributions of diurnal and nocturnal pollinators in female reproductive success of plants are not constant, and might fluctuate in space and time (Morse and Fritz 1983, Schemske and Horvitz 1984, Waser et al. 1996, Gómez and Zamora 2006). At our study site, nocturnal moths play a minor role in propagation success of *J. curcas*, which implies that the plant producing small amounts of nectar in the night could only attract small number of moths. Dehgan and Webster (1979) described the flowers of *J. curcas* in some place might emit sweet, heavy perfume at night and copious nectar, which might be more attractive to the nocturnal pollinators, such as moths, than at our site. The plant is widely distributed in tropical and subtropical areas worldwide (Heller 1996) and originated from Central America (Fairless 2007), where nocturnal pollinators are more important than at present site (Corlett 2004). In that case, abundant nocturnal pollinators might be attracted to visit the flowers of *J. curcas* and play a greater role in female reproductive success than at our site.

In conclusion, our study shows that diurnal visitors contribute more than nocturnal visitors to the female reproductive success of *J. curcas*. However, if diurnal visitors are lacking or removed in this species at this location, then nocturnal visitors will still play a major role in pollination. In areas where natural pollinators are lacking, honey bees might be artificially placed to provide pollination because $\approx 30\%$ of visits are made by honey bees.

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