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POTENTIAL POLLINATORS AND FLORAL VISITORS OF INTRODUCED TROPICAL BIOFUEL TREE SPECIES *Jatropha curcas* L. (Euphorbiaceae), IN SOUTHERN AFRICA

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ABSTRACT

Jatropha curcas L. is a tropical tree belonging to the Euphorbiaceae family with oil-rich seeds that are currently used as a source of biodisel. A better knowledge of the reproductive ecology is imperative for yield optimisation and invasiveness risk assessment. Insight in the diversity and movement of potential pollinators, forms an important aspect of the needed knowledge. In this paper, we report on *J. curcas* flower visitors observed in two Southern African countries, Zambia and Malawi. A total of 41 insects and 2 Arachnida in Zambia and 29 insect species in Malawi visited *J. curcas* flowers. Diptera and Hymenoptera were the largest groups. The most abundant insect visitors were *Apis mellifera* and *Chrysomya chloropyga*. *Apis mellifera* visited more inflorescences within short periods than *C. chloropyga,* and showed a frequent appearance in both sites. Therefore, *Apis mellifera* seems to be more important in the pollination process of *J. curcas* in the Southern African region.

Key Words: *Apis mellifera*, biodisel, *Chrysomya chloropyga*

RÉSUMÉ

Le *Jatropha curcas* L. est un arbre tropical appartenant à la famille des Euphorbiaceaes avec de graines riches en huile couramment utilisées comme source de “biodisel”. Une meilleure connaissance de l’écologie reproductive est impérative pour l’optimisation du rendement et l’évaluation du risque d’invasivité. La compréhension de la diversité et du mouvement des pollinateurs potentiels est un aspect important de la connaissance désirée. Cet article est un rapport sur les visiteurs des fleurs de *J. curcas* observés dans deux pays sud africains, la Zambie et le Malawi. Un total de 41 insectes ainsi que 2 Arachnida en Zambie et 29 espèces d’insectes au Malawi avaient visité les fleurs de *J. curcas.* Les Diptères and Hymenoptères étaient les groupes les plus larges. Les insectes visiteurs les plus abondants étaient *Apis mellifera* et *Chrysomya chloropyga*. *Apis mellifera* avait visité plus d’inflorescences pendant des courtes périodes que *C. chloropyga,* et avait manifesté une apparition fréquente dans tous les deux sites. Par conséquent, *Apis mellifera* paraît être le plus important dans le processus de pollinisation de *J. curcas* dans la région sud africaine.

Mots Clés: *Apis mellifera*, biodiesel, *Chrysomya chloropyga*

INTRODUCTION

The development of bioenergy system has attracted considerable public, commercial and scientific attention as a potential solution for the global climate and energy crisis (Rajesh *et al*., 2008; King *et al*., 2010). *Jatropha curcas* L. is a biofuel crop, widely cultivated in Africa, Central and South America, India and Southeast Asia (Katembo and Gray 2007; Maes *et al*., 2009), mainly because of the high quality oil it produces and its ability to reclaim dry, marginal and degraded areas (Achten *et al*., 2008; Achten *et al*., 2010a). It is a tropical, drought resistant, stem succulent tree originating from continental central America (Kaushik *et al*., 2007; Kumar and Sharma, 2008; Maes *et al*., 2009; Trabucco *et al*., 2010). *Jatropha curcas* is monoecious and flowers during rainy seasons in racemose inflorescences, which follow a dichasial cyme pattern; wherein both male and female flowers develop (Carels, 2009; Achten *et al*., 2010b).

Despite the huge interest in *J. curcas*, scientific information on the breeding and domestication of the species is limited (Achten *et al*., 2010a; 2010b). In Africa, there are only a few publications on the pollination ecology of the species. Examples are those of the Banjo *et al*. (2006) in Nigeria on insects associated with *J. curcas*, and Abdelgadir *et al*. (2009) on honey bee effectiveness in the pollination of *J. curcas*. Knowledge on pollination processes of plant species is a crucial component of regeneration ecology, as pollination is a key process in the sexual reproduction of most angiosperms and can directly affect the plant reproduction success (Blancafort and Gomez, 2005).

For a monoecious plant like *J. curcas*, pollinators are essential for successful seed production (Santos *et al*., 2005). Wind pollination from plant to plant is minimal (Chang-wei *et al*., 2007) because of the adhesive nature of *J. curcas* pollen and the smoothness of the stigma, which is typical of plants pollinated by animals. Moreover, reproductive success of *J. curcas* is not only dependent on the presence of pollinators, but also on the species composition of the pollinator community (Herrera, 2000; Abdelgadir *et al*., 2009). According to Rianti *et al*. (2010), a variety of factors affect pollination: pollinator visit activities on flowers, the compatibility of animal-pollinator's morphology with flower phenology, visit duration and visit frequencies. But the *J. curcas* flower visitor species and their foraging behaviour are only marginally researched. So far, there are no pollinator recommendations for *J. curcas*, though it is of great importance to determine how these vital pollination services will be provided for the plant (Vaknin, 2012). This will fill the knowledge gap in regeneration ecology and design, suitable breeding and development strategies to maximise *J. curcas* productivity in specific growing zones.

The objective of this study was to determine the main visitors of *J. curcas'* inflorescences; and their visit frequence of the inflorescence; kind of forage they look for; and how much time *A. mellifera* and *C. chloropyga* spend per inflorescence and per tree.

MATERIALS AND METHODS

Study site. The study was conducted in Zambia and Malawi in Southern Africa. In Zambia, the study was conducted in the Chongwe district, Lusaka province, from the end of November till the end of December 2009; on a 5 year old *J. curcas* plantation, of 0.5 ha with 2 m × 2 m spacing. This site (15°22'09" S; 28º27'33" E) has mean altitude of 1160 m above sea level (a.s.l.). The area has a long rainy season (November to April) and receives mean annual rainfall of 802 mm, most of which occurs between November and March. The mean annual maximum and minimum temperature are 26 and 14°C, respectively. The geology of the area is dominated by recently extended alluvium or colluvial quaternary deposit formations with well drained shallow to moderately shallow sandy clay loam Ferric Luvisols based on the FAO classification system (Chinene, 1988).

In Malawi, the study was conducted in the Balaka district, from beginning of December to end of December, 2010; in a 5 years old *J. curcas* plantation of 4 ha with a spacing of 2 m× 3 m. This site (15°12'20" S; 29º08'36" E) has a mean altitude of 480 m a.s.l. It experiences a tropical climate with three main seasons; cold dry, hot dry and hot wet, ranging respectively from April to July, August to October and November to

March (EADM, 2006). The mean annual rainfall for 2009 and 2011 was 620 mm, with a mean annual minimum temperature of 19 °C and mean annual maximum of 29 °C. The soil of the area is dominated by Haplic Lixisols and Vertisols based on the FAO soil classification system (EADM, 2006).

Data collection and analysis. At both study sites, ten *J. curcas* trees were selected randomly. The species diversity which visited *J. curcas* inflorescence, their number and type of foraging they collect were observed for a period of 10 inconsecutive days during the respective peak flowering periods (3 to 14 December, 2009 in Zambia; December 9 to December 27, 2010 in Malawi). The minimum and maximum temperature of the observation month was 17.9 and 29.2 °C, respectively, with a total rainfall amount of 188.7 mm in Zambia site. In Malawi site, the minimum and maximum temperature of the month was 23 and 38.7 °C, respectively, with a total rainfall amount of 116.2 mm.

Observations were made in the morning (07:00 - 12:00 AM) and in the afternoon (13:00 - 17:00 PM). Each tree was observed for 20 minutes in the morning (200 minutes for 10 trees) for every observation day and 20 minutes in the afternoon (200 minutes for 10 trees) by standing quietly next to the target tree and recording the visiting species, and counting their visit frequence. Two persons stood on opposite sides of the tree for this purpose.

In cases where we could not identify the visitor in the field, it was collected using white net trap for identification. The collected specimens were kept in 70% alcohol solution. Most of the collected flower visitors were identified to family level using Picker *et al*. (2004) and by insect experts from D1 Oils plant science Limited Company, Zambia, and the University of Zambia. For each flower visit, we also recorded the visitor foraging for pollen, nectar, both or other floral parts.

For the observations in Zambia, we determined the visiting time per inflorescence for the 2 most abundant visitors. Each time when the most abundant visitor came; we timed how long it stayed on an inflorescence and on a tree, for 104 random observations made during seven consecutive days.

Statistical analysis. Descriptive and non-parametric statistical analyses were carried out using SPSS 15.0 (SPSS Inc., Chicago, IL). Simpson's index of diversity (SID) was calculated for each site using the formula (Simpson, 1949):

$$SID = 1 - \left[\frac{\sum n_i (n_i - 1)}{N(N-1)} \right]$$

Where, n_i = number of individuals of each species observed, N = total number of all species observed and SID = Simpson's Index of Diversity, which is a measure of diversity which takes into accounts both richness and evenness. To compare *J. curcas* flower visitors' frequency in the morning and afternoon, related non-parametrical Wilcoxon Matched Pairs test was used. Visit frequency based on order was analysed using Friedman Test, considering one Arthropod's order may visit different inflorescence and trees. To compare inflorescence and tree handling time of the two most abundant insect visitors non-parametric Kruskal-Wallis test (KW) used.

RESULTS

Main inflorescence visitors. *Jatropha curcas* inflorescences were visited by a variety of insects and a few arachnids. In the Chongwe site (Zambia), a total of 43 insect species and 2 spider species were observed visiting the flowers (Table 1). In Balaka site (Malawi), only insect species visited *J. curcas* inflorescences. In total, 29 insect species were observed (Table 2). The Simpson's diversity index value showed that flower visitors in Malawi (0.79 and 0.71morning and afternoon, respectively) were more diverse than Zambia (0.55 and 0.38 morning and afternoon, respectively).

Frequency of visits to the inflorescence. In Zambia, there was a significant difference in visit frequency between the different orders, (χ^2 = 57.805, P < 0.001) (Table 1). In general, visit frequencies did not significantly differ between morning and afternoon (Z = -0.167, P = 0.867). Dipterans were the largest group of visitors, with 3,796 individuals (accounting for 74 and 87%, respectively in the morning and afternoon

TABLE 1. Diversity of insect and arachnida species visited *J. curcas* inflorescence in Chongwe district, Zambia

Class	Order	Families	Species richness	Forage collected	Time of observation					
					Morning			Afternoon		
					n (#)	n (%)	Av	n (#)	n (%)	Av
Insecta	Hymenoptera	10	13	P & N	542	22.55	5.42	221	9.53	2.21
	Diptera	8	10	N	1770	73.66	17.70	2026	87.36	20.26
	Hemiptera	4	4	P, N & Pe	55	2.29	0.55	51	2.20	5.10
	Lepidoptera	4	8	N	10	0.42	0.10	5	0.22	0.05
	Coleoptera	5	5	W	22	0.92	0.22	16	0.69	0.16
	Mantodea	1	1	N	1	0.04	0.01	0	0.00	0.00
Arachnida	Araneae	1	2	P & N	3	0.12	0.03	0	0.00	0.00
Total		33	43		2403			2319		

Note: n (#) = Total individuals of order; n (%) = Total individuals of order in percent; Av = Average individuals of order per observation periods (200 minutes) per tree; P = Pollen; N = Nectar; Se = Sepal; Pe = Petal; W = The whole part of the flower. The observation was carried out in the morning between 07:00 - 12:00 a.m. and in the afternoon 13:00 - 17:00 p.m.

TABLE 2. Diversity of insect species visited *J. curcas* inflorescence in Balaka district, Malawi

Class	Order	Families	Species richness	Forage collected	Time of observation					
					Morning			Afternoon		
					n (#)	n (%)	Av	n (#)	n (%)	Av
Insecta	Hymenoptera	8	9	P & N	952	49.87	9.52	993	60.62	9.93
	Diptera	6	6	N	664	34.78	6.64	421	25.70	4.21
	Hemiptera	3	4	P, N & Pe	143	7.49	1.43	133	8.12	1.33
	Lepidoptera	4	6	N	41	2.15	0.41	17	1.04	0.17
	Coleoptera	2	2	W	14	0.73	0.14	7	0.43	0.07
	Odonata	1	1	N	2	0.10	0.02	0	0	0
	Orthoptera	1	1	W	93	4.87	0.93	67	4.09	0.67
Total		25	29		1990			1638		

Note: n (#) = Total individuals of order; n (%) = Total individuals of order in percent; Av = Average individuals of order per observation periods (200 minutes) per tree; P = Pollen; N = Nectar; Se = Sepal; Pe = Petal; W = The whole part of the flower. The observation was carried out in the morning between 07:00 - 12:00 a.m. and in the afternoon 13:00 - 17:00 a.m.

observations) of all collected individual visitors belonging to 10 species. The Hymenopterans were the second group, consisting of 763 individuals, accounting for 23 and 10% respectively, in the morning and afternoon observations of the total visitors. This order included 13 species (Table 1). Of the individual visitors, *C. chloropyga* was the most abundant (64.67% in the morning and 78.57% in the afternoon); followed by *A. mellifera* (18% in the morning and 6% in the afternoon).

In Malawi, the visitor frequency was significantly different between orders (χ^2 = 19.835, P < 0.001). Similar to Zambia, there was no difference in morning and afternoon frequencies (*Z* = -1.098, P = 0.272). The observation showed that the Hymenopterans were the most frequent visitors (1945 individuals), representing 49.87 and 60.62% of morning and afternoon visits, respectively (Table 2). *Apis mellifera* (Hymenoptera) was the most dominant visitor (40.18% in the morning and 50.49% in the afternoon).

Foraging behaviour. Insect and arachnid groups gathered pollen and/or nectar. In Zambia, about 29 insect species (67% of the observed species) foraged on nectar; and 6 insect and 2 arachnid species accounting 19% foraged on both nectar and pollen (Table 1). The remaining 6 (14%) insects foraged on the whole floral part. In Malawi, 52% and 28% insect species foraged for nectar and both nectar and pollen, respectively. Six insect species foraged on the whole floral parts, accounting 20% of the total observed insect species.

All Lepidoptera and Diptera foraged on nectar; during nectar collection most Diptera species carried pollen on their body hairs, and then removed it from hairs with their legs. They preferred to move slowly between flowers on the inflorescence and between inflorescences. Beetles (Coleoptera) and bugs (Hemiptera) moved between flowers of the same inflorescence. Most beetles and hemipterans showed two types of foraging behaviour; ingesting pollen and sucking nectar and sometimes eating the whole flower (Tables 1 and 2). From the Hymenoptera group, bees collected pollen onto the scopa of the hind femur; and sucked nectar at the base of *J. curcas* flowers.

Inflorescence handling time. The inflorescence handling time recorded for the two most important insect species (*A. mellifera* and *C. chloropyga*) in Chongwe, Zambia was significantly different (KW, χ^2 = 76.02, P < 0.001 and KW, χ^2 = 72.96, P < 0.001 on a per inflorescence basis and per tree basis, respectively). The highest inflorescence handling time was observed for *C. chloropyga* (118±12 sec/inflorescence and 198±12 sec/tree). Inflorescence handling time of *C. chloropyga* varied significantly between days (KW, χ^2 = 20.01, P = 0.003). The mean time spent by *A. mellifera* to handle a single inflorescence and tree was 8±1 seconds and 22±2 seconds, respectively.

DISCUSSION

***Jatropha curcas* inflorescence visitors and visit frequency.** Based on the abundance and frequency, Hymenoptera and Diptera insect species were the most abundant and frequent visitors of *J. curcas* inflorescences (Tables 1 and 2). Most of them were also reported as *J. curcas* flower visitors by Banjo *et al*. (2006) in Nigeria, Raju and Ezradanam (2002) in India and Rianti *et al*. (2010) in Indonesia. This shows that these insect orders are attracted by *J. curcas* floral rewards (nectar and pollen) and they probably play a large role in the transport of pollen between flowers, inflorescences and trees.

Flies were the most abundant flower visitors in Chongwe, Zambia and honey bees in Balaka, Malawi. The high number of flies (*C. chloropyga* and *M. domestica*) in Chongwe, Zambia, might be due to the presence of settlements and waste disposal pits near to this particular plantation. The honey bees in Zambia might be from managed colonies of the surrounding farms. In Malawi, the plantation was surrounded by Miombo woodland and honey bees could be either from wild colonies or coming from the nearby villages about 5 to10 km away from the plantation. This has two implications; the first one is honey bees are very much attracted to *J. curcas* flowers and they could be important in successful pollination of *J. curcas*. The other implication is the attractiveness of *J. curcas* flowers to local pollinators might have adverse effect to native flowering plants. This could provoke pollinator competition between native flora and introduced biofuel crops through reducing pollinator visitation rates, or through increased heterospecific pollination of native flowers. If this negative phenomenon occurs among native and new *J. curcas* plantations, it might cause reductions in pollen quantity and pollen quality and final seed set of native plants.

Forage behaviour. The majority of the recorded flower visitors used nectar of *J. curcas* flowers as their food source (Tables 1 and 2). Both male and female flower bases secrete nectar with a nectar production of 4.54±0.82 µl in female flowers, and 1.92±0.44 µl in male flowers in 1200 hr (Bhattacharya *et al*., 2005). To collect nectar and pollen from *J. curcas* flowers, most insects usually perched on the shiny, yellowish petals,leading to their bodies getting covered with pollen. Then pollen was available to pollinate female flowers during the next visit of the insect. This would facilitate pollen transfer from one flower to the other, and thus may improve pollination success and final fruit set.

From their foraging behavior, Raju and Ezradanam (2002) explained the importance of honey bees and flies as effective pollinators of *J. curcas* plantations in India. *Eumenes* and *Vespa* wasps and beetles are not effective pollinators as they are considered to be nectar robbers (Bhattacharya *et al*., 2005) and sometimes the beetles forage on the whole flower parts. In Indonesia, flies were not considered effective pollinators possibly because of their infrequent visits (Rianti *et al*., 2010). We observed that during nectar collection, the flies positioned their bodies away from the anthers, but the legs did make contact with the anthers. During this process, pollen may be relocated to stigmas through contact with their legs.

Butterflies also visited many inflorescences; whereas most hemipterans and coleopterans were restricted to a few inflorescences on the same tree. From this study, butterflies are not considered consistent potential pollinators, because of their infrequent visits, but probably they can occasionally function as pollinators. From the Lepidoptera order, two moths in the Geometridae and Lymantriidae family, could also be *J. curcas* flower visitors during the night. Most hemipterans, coleopterans and orthopteran ate the sepals, petals or the whole flower of *J. curcas* and were considered in this study as *J. curcas* pest species (such as *Zonocerus elegans*, *Galidea dregii*, *Rhinocoris neavii*, *Cletus* sp., and *Anoplocnemis curvipes*). High *J. curacs* fruit abortion rates by fruit-feeding bugs, *Calidea dregii* and Elegant grasshopper (*Zonocerus elegans*), was also reported by Negussie *et al*. (2013) and Grimm (1999). We suggest further investigations in identifying, monitoring and management of these pests to avoid the damage and increase *J. curcas* productivity in the tropics.

Inflorescence handling time. Based on inflorescence handling time study, bees collected pollen and nectar inconsistently, with short durations per inflorescence; while flies spent long periods in a single inflorescence. We observed also inflorescence handling time to differ over the seven consecutive observation days for *A. mellifera* and *C. chloropyga*. This might be as a result of pollen and nectar availability difference between the first and later days. Resource availability on *J. curcas* flowers determines flower visit duration and frequency (Bhattacharya, *et al*., 2005). Moreover, most flowers offer only small quantities of nectar as a strategy to keep pollinators moving between trees and thereby maximise pollen transfer (Spaethe *et al*., 2001).

CONCLUSION

Considering the visitor abundance, frequency and foraging characteristics, insects play a crucial role in the pollination ecology of *J. curcas* in the study sites. Honey bees are probably also more efficient than *C. chloropyga* as pollinators of *J. curcas*, as they visit many inflorescences and they have specialised scopae to forage for pollen; in contrast with the Diptera that lack these features. Considering their abundance, visit frequency and foraging characteristics, honey bees might play a crucial role in the pollination ecology of *J. curcas.* Combining beekeeping with large or small scale *J. curcas* cultivation may provide dual advantages of encouraging the pollination process in the plantation and honey production as an additional income for the growers.

We recommend further research on pollination interactions between Jatropha plantations and native flowering plants in the study area. *J. curcas* flowers bloom in mass and are intensively visited by many pollinators, indicating that it might have the potential to affect pollination of other native plant species. Moreover, the flowering season of mature *J. curcas* (end of October to beginning of January) coincides with the flowering of most important Miombo native shrubs and trees (such as, *Julbernardia globiflora*, *Julbernardia paniculata*, P*terocarpus angolensis*, *Isoberlinia angolensis*, *Parinari curatellifolia*, *Uapaca kirkiana*) in Zambia and Malawi.

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