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Full Length Research Paper

Pollinator diversity, behaviour and limitation on yield of karela (*Momordica charantia* L. Cucurbitaceae) in Western Kenya

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Inadequate pollination is a major constraint to crop yield. *Momordica charantia* L. is a fruit crop of economic interest in Kenya. Pollination ecology, pollinator diversity and their behaviour were studied in Western Kenya. Pollination treatments included insect exclusion, open pollination (unrestricted insect visits), hand cross-pollination and pollen augmentation. Yield components from treatments were compared to identify the pollination requirements of this crop. Flowering started 45 days after germination with the staminate flowers appearing first followed by rewardless pistillate flowers. The ratio of pistillate to staminate flowers was 1: 13. Pollinator species included honey bees (*Apis mellifera*), *Plebeina hildebrandti*, *Lasioglossum* sp. and carpenter bees (*Xylocopa* spp). Fruit set and yield were pollen limited as all bagged flowers were aborted. Fruit set under natural pollination was very low and this revealed the degree of pollen limitation in *M. charantia*. Low fruit set was consistent with observation of high discrimination against pistillate flowers amongst potential pollinators. Smaller bees belonging to families Apidae (*Plebeina hildebrandti)* and Halictidae (*Lasioglossum* sp.) were the most important pollinators. These observations highlight the importance of (1) a diverse fauna of wild bees and (2) the potential of meliponiculutre in the increasing the yield of *M. charantia* in Kenya.

Key words: *Plebeina hildebrandti, Lasioglossum,* discrimination, deceit pollination, yield.

INTRODUCTION

A recent review on the worldwide dependence of crops on pollinators showed that 87 out of the 124 leading food crops are dependent on animal pollination (Klein et al., 2007). In the tropics, insect pollination increases fruit and seed production in 70% of the crops (Roubik, 1995). Lack of pollination therefore, can be a major limiting factor to high fruit seed yields and its quality. There is a growing literature on the decline of pollinators worldwide that has prompted a growing interest in the importance of pollinator diversity in both natural and crop ecosystems (Buchmann and Nabhan, 1996; Allen-Wardell et al., 1998; Kevan, 1999). For example, monoecious plants bearing both pistillate and staminate flowers on the same plant but at different locations. For effective pollen transport in such crops, pollinators are very essential as active selfing is not possible (like it is in hermaphroditic flowers). In most of these species, staminate flowers offer nectar and pollen while the pistillate flowers offer only nectar as floral rewards to pollinators (Free, 1993). For some plant species, for example, cucumber (*Cucumis sativus),* squash *(Cucurbita pepo)* and zucchini (*Cucurbita maxima)*, pistillate flowers produce more nectar per flower than the staminate flowers and therefore attract pollinators differently (Nepi et al., 1996). Moreover, in others, for example, luffa (*Luffa acutangula*), bottle gourd (*Lagenaria vulgaris* and karela (*M. charantia*), pistillate flowers are rewardless (Bahadur et al., 1986). These pistillate reward-

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less flowers mimic the staminate flowers and are in most cases pollinated by deceit (Baker, 1976; Dafni, 1984).

Though variable amongst pollinator species, discriminat-ion against rewardless pistillate flowers has been demonstrated in a number of species including papaya (*Carica papaya*) (Baker, 1976), squirting cucumber (*Ecballium elaterium*) (Dukas, 1987), and several *Begonia* species (Ågren and Schemske, 1991; Schemske et al., 1996). Ågren et al. (1986) found bumble bees to discriminate against the rewardless pistillate flowers of cloudberry (*Rubus chamaemorus*) while syrphid flies did not. Dukas (1987) studied the frequency visits of various bee species to pistillate and staminate flowers of *E. elaterium* and found that *A. mellifera* discriminated between the two genders of flowers and paid relatively fewer and shorter visits to the pistillate as compared to the staminate flowers. Thus, discrimination against pistillate flowers can indeed affect reproduction of animal-pollinated plants and may be especially severe in crops with rewardless pistillate flowers. No study has however, elucidated how this discrimination affects the female reproductive success, and crop yield of an agricultural crop like *M. charantia* with rewardless pistillate flowers.

M. charantia is a tropical and sub-tropical vine of the family Cucurbitaceae, widely grown for edible fruit, which is among the most bitter of all fruits. Its centre of origin is believed to be in Eastern India and Southern China but high species diversity occurs in Africa (Joseph, 2005). Other common names for the plant and its fruit include: bitter melon, karela, bitter gourd, balsam pear, ampalaya, amongst others. It is a vegetable crop of high economic value in India and Africa (Palada and Chang, 2003) and in particular of interest amongst the Asian community in Kenya (HCDA, 2009). It has a high nutritive value especially, high ascorbic acid, iron (Behera, 2004) and also medicinal properties against a wide range of diseases, including improving the immune system of HIV-AIDS patients (Njoroge and van Luijk, 2004). In spite of the potential economic and medicinal importance of the crop, yields have been low: 12,000 kg ha^{-1} in 2008 and 6,400 kg ha^{-1} (HCDA, 2009). Other factors for example, reduced acreage under production have been linked to this decline without even considering insufficient pollination. Earlier studies have focused on the agronomic practices (Palada and Chang, 2003), medicinal value (Njoroge and van Luijk, 2004) and only recently its pollination biology (Deyto and Cervancia, 2009). *M. charantia* bears both staminate and pistillate flowers on the same plant but at different locations and relies heavily on pollinators for fruit set (Behera, 2004). Palada and Chang (2003) gave a female to a male flower ratio of 1: 25 while Deyto and Cervancia (2009) reported a ratio of 1: 19. Female flowers are rewardless but often bear a striking resemblance to the polleniferous and nectari-ferous male flowers.

Studies on *M. charantia* pollinator diversity and their influence on the yield of *M. charantia* are scanty (Free, 1993). Honey bees (Roubik, 1995) and halictids (Grewal and Sidhu, 1978) have been reported as the principal pollinators of cucurbits including *M. charantia* in Tropical America and India respectively. Other important pollinators include *Apis florea, A. cerana* and *A. dorsata* (Apidae) in India (Behera, 2004), *Diabrotica speciosa* (Coleoptera, Chrysomelidae) in Brazil (Lenzi et al., 2005) while Deyto and Cervancia (2009) recorded *A. mellifera, A. cerana, Trigona* sp. (Apidae) and *Halictus* sp. (Halictidae) foraging on *M. charantia* in Philippines. A poor fruit set of 22% has been recorded under natural pollination in India (Mishra and Sahoo, 1983) and hand pollination was recommended to improve fruit set. Deyto and Cervancia (2009) recorded higher fruit set of 78% in natural pollination in Philippines but it was not clear, if the floral visitors showed any preference for the staminate flowers and how that influenced the final crop yield. Recent studies on the genus *Momordica* by Joseph (2005) and Behera et al. (2010) suggested that for a commercial fruit and seed production, pollination management for this crop is essential and the use of hand pollination or the introduction of honey bee colonies in enclosures in India is recommended. The increasing demand for these fruits amid poor yields in Kenya has led to the need of understanding the pollination ecology, pollinator diversity and behaviour, and their influence on the yield and conservation management of this crop. This paper therefore, aims to explain these aspects as well as yield improvement strategies. To achieve this, the following questions were addressed: How do floral display and flowering time differ between male and female flowers of *M. charantia*? Who are the floral visitors of *M. charantia* in Kakamega, Kenya? Do floral visitors discriminate between male and female flowers and do they differ in terms of their behaviour and the time spent on the different flower sexes? Is the female reproductive success and the subsequent crop yield pollinator limited as a result of this discrimination?

MATERIALS AND METHODS

Study site

The study was conducted within the farmlands around Kakamega Forest located between latitudes 00° 08′ 30.5″N (41 236 in UTM 36 N) and 00° 22′12.5″N (15 984) and longitudes 34° 46′ 08.0″ (696 777) and 34° 57′ 26.5″ E (717 761) and altitude of about 1500 to 1700 m (KIFCON, 1994). The farmlands of Kakamega forest consist of rich agricultural soils, and the high rainfall of about 2000 mm is well distributed throughout the year. The two peaks occur within April to May (long rains) and October to November (short rains) with mean monthly temperatures ranging from 11 to 29°C with an average daily temperature of 22°C (Jaetzold and Schmidt, 1982). The land use in this area is mainly small scale farming with sugarcane being the most dominant cash crop with other crops being maize, beans, pumpkins, a few vegetables and fruits that are mainly for the farmers' house hold food requirements. Beekeeping in Kakamega district is conducted on a small-scale basis only, without high commercial intention. Farmers construct hives, but do not rear queens or colonies, and, thus, rely on feral honeybee colonies to enter the hives (Hagen and Kraemer, 2010). Most of the

crops grown rely either on these feral bees or the many solitary bees for pollination.

Field preparation

M. charantia seeds were planted on a 8 × 8 m plot. At planting, inorganic fertilizers, DAP was applied at a rate of 100 kg ha^{-1}. The first planting was done in April 2009, and the second planting in April 2010, at a spacing of 90 × 60 cm, sowing three seeds per hole. After germination, thinning was done leaving only one plant per hole, giving approximately 120 plants in the experimental block. Trellising using sticks and straw was done to support the plants. Weeding was done thrice using hand held hoes.

Flower phenology and nectar production

During the flowering period, numbers of open pistillate and staminate flowers per day were counted and recorded for the entire 70 observation days in years, 2009 and 2010. The flowers only last for a day. Numbers of pistillate and staminate flowers was not counted per plant but per experimental plot. Observations on the floral colours, shape and the number of days the flowers remained open were noted. Nectar production was measured every hour from 0900 till 1400 h on five randomly selected staminate flowers. Five flowers were selected every hour on each day for five days. To confirm the absence of nectar on pistillate flowers, flowers buds were bagged an evening before flower opening to completely exclude any foraging prior to the experimental nectar collection. Nectar was extracted using 2 µl micro capillary tubes. The length of the nectar in the column was noted and the volume calculated according to Cruden and Hermann (1983). Nectar was then deposited on the low-volume field hand held refractometer prism (0 to 50%, Bellingham and Stanley, Norcross, Georgia, USA) for the measurement of solute concentration (percentage sucrose equivalents on a mass basis).

Pollinator foraging behaviour and visitation frequency between the two flower genders

Behaviour of flower visitors of *M. charantia* was observed between 0900 to 1400 h for 70 days in both 2009 and 2010 observation periods, amounting to 350 observation hours. Total number of foragers was noted at every one (1) h interval in the whole plot. Careful observations were made while walking along the crop rows on approaching/landing foraging insects, without disturbance, the forager was followed and records made on the sex of the visited flower, and the number of visited flowers, using a hand held tally counter. Furthermore, the total time the flower visitor took on the plot was taken using a stop watch which was stopped as soon as the forager flew away from the plot. Foraging behaviour (nectar or pollen collector) of the forager on the flowers was also noted. Direct attempts were made to identify the insect species visiting the flowers, but foraging species identification was always difficult in the field, therefore, voucher specimens were collected, identified and deposited at the Invertebrate Zoology Laboratories (IZL) of the National Museums of Kenya, Nairobi (NMK).

Pollination treatments

For comparison between different pollination treatments in other words, “pollinator exclusion”, “open pollination”, “hand cross pollination” and “pollen augmentation” a Complete Randomised Design (CRD) was used. In the “pollinator exclusion” treatment, pistillate flowers were bagged with 1 mm mesh netting and labelled, while in the open pollination treatment, pistillate flowers were labelled and left open for unrestricted floral visits throughout the treatment period. To estimate the maximum possible fruit and seed set when pollen is not limiting, pistillate flower buds were bagged on the evening before opening and the next day after flower opening between 0900 and 1100 h, the stigma was dusted with pollen collected from 2 to 3 staminate flowers from a different plant. The flowers were labelled and bagged to exclude any further visits. The need for pollen augmentation in natural pollination situation was investigated by performing hand cross pollination (as in the treatment previously described) but the flowers was further left open for more visits by pollinators. To compare the pollinator effectiveness of the different floral visitors, single visits were allocated. In this case, pistillate flower buds were bagged an evening before flower opening and the next day after flower opening between 0900 and 1100 h, they were exposed to one single visit by a floral visitor. On visiting the exposed flower, the flower visitor was identified (if possible), the flowers labelled and bagged with 1 by 1 mm netting to exclude further visits. The bags were removed after fruit set to allow for fruit development. Fruit set was recorded 10 days after the treatments were done. Fruits were harvested after 20 days from the treatment initiation and the fruit weights, length of fruit, number of mature seeds per fruit as well as, the weight of seeds per fruit were recorded. Only mature seeds were counted in all the treatments.

Statistical analysis

Frequency of visits to staminate and pistillate flowers for the all the visitors were compared using pivot table in Microsoft Office Excel 2007. Nectar volumes and concentration at the different times of the day, and the time taken by the floral visitors during foraging were analysed for staminate and pistillate flowers using univariate Analysis of Variance (ANOVA) with General Linear Models procedure (IBM® SPSS® Statistics version 19) with time (seconds) on the flower as the response variable and pollinator species as fixed variable. To analyze the effect of floral visitors on the yield components of *M. charantia*, ANOVA was performed with GLM, with fruit weight, length of fruit, number of mature seeds per fruit and weight of mature seed per fruit as dependent variables and pollination treatments (open, hand cross and pollen augmentation) as independent variables. Pollinator exclusion treatments all aborted and were therefore excluded from this analysis. Significant differences between the treatments were performed at 95% Confidence Interval (C. I) and the treatment means compared using the Student-Newman-Keuls (S-N-K) post hoc test.

RESULTS

Flower phenology and nectar production

Flowering started approximately 45 days after seed germination. Staminate flowers bloomed earlier than pistillate flowers. Though not measured in the current study, staminate and pistillate displayed some resemblance (corolla shape and colour), except that the staminate flowers appeared bigger in size than the pistillate flowers. The staminate flowers have fleshy anthers covered with orange-yellowish coloured pollen borne on somehow thick fused filaments. Below the thick filaments are tiny openings that lead to the nectary. The female flowers are easily recognized by the three lobed fleshy stigmas that are greenish in colour and borne on a thick style with an

ovary at the pedicel. They do not offer any reward to flower visitors. Staminate flowers are opened early in the morning from around 0700 h, while the female flowers are opened 2 h later around 0900 h. Flowers of the two sexes have a long period of overlap in flowering. In both cases, the flowers were open for only one day. Staminate flowers wilt off by 1200 h and fall off by late afternoon. Female flower petals start to close late in the afternoon at about 1400 h with the petals falling the next day. Successful pollinated female flowers start to set fruit within five days while in un-pollinated flowers, the ovaries were observed to turn yellowish to brown before they finally dried up.

Pistillate flowers were lower in numbers compared to the male flowers. The total number of staminate flowers in 2009 was 9930 and the number of pistillate flowers was 758, giving a mean of 230.9 ± 13.67 staminate and 17.6 ± 1.1 pistillate open flowers per day in the experimental plot while in 2010, staminate flowers were 1460 and pistillate 109, giving a mean of 50.3 ± 16.64 staminate and 3.8 ± 1.34 pistillate opened flowers per day in the experimental plot. The ratio of female to male flowers was 1:13 in both 2009 and 2010 in the experimental plot.

Results from this study revealed that female flowers had no nectar while the staminate flowers produced nectar. Nectar volumes were high in the morning hours and decreased towards late afternoon ($F_{5, 91}$ = 8.219, P<0.001). The percent sucrose concentration w/w in the nectar was constant with mean concentrations of 25.0 ± 0.55% sucrose w/w throughout the day ($F_{5, 75}$ = 0.656, P>0.05).

Pollinator foraging behaviour and visitation frequency between the two flower genders

Eleven insect species representing two orders (Hymenoptera and Diptera) were recorded (Table 1), while honey bees (*A. mellifera*), *P. hildebrandti* and *Lasioglossum* sp. were frequent visitors, carpenter bees (*Xylocopa* spp.) were considered to be of moderate frequency to the flowers of *M. charantia*. Few observations of *Hypotrigona gribodoi* and only a single record of a muscid fly, *Amegilla* sp. and *Megachile* sp. were recorded visiting male flowers only. They were considered to be of negligible reproductive importance and were excluded in the forager behaviour and visitation analysis. Floral visitors were most likely to visit more staminate flowers per bout as compared to pistillate flowers. It was unlikely that they made more than one visit to pistillate flowers in any single foraging bout. In most of the cases, a bee landing on a pistillate flower flew away from the study plot without visiting another flower (staminate or pistillate). In both observation years (2009 and 2010) the proportion of pistillate flowers that received visits by pollinators remained below 1% of the total recorded visits by pollinators. In 2009, the ratio of visited pistillate to staminate was 1: 430 while in 2010 was 1: 227. In fact, pistillate flowers received the recorded visits only by chance. *A. mellifera*, *P. hildebrandti, X. flavorufa* and *X. inconstans* visited pistillate flowers, but in very negligible frequencies (Table 1).

Foraging time had an influence on the number of staminate flowers visited in each single foraging bout by the different visitor species ($F_{29, 1043}$ = 1.590, N = 1084, P≤0.05). For larger *Xylocopa* spp. the number of visited flowers increased significantly in the afternoon. *X. nigrita* visited 3.6 ± 0.66 staminate flowers at 0900 and 6.0 ± 1.0 at 1300 h. Likewise, *X. flavorufa* visited 4.4 ± 0.52 at 0900 and 5.1 ± 0.56 at 1300 h. Other small sized pollinator species; *Lasioglossum* sp. and *P. hildebrandti* had no change in the number of staminate flowers visited with time of the day. Number of staminate flowers visited per foraging bout by each pollinator species were significantly different ($F_{6, 1043}$=56.602, N=1084, P≤0.001) in 2009 and ($F_{6, 436}$ = 37.093, N = 462, P≤0.001) in 2010. Bigger bees (*Xylocopa* spp.) visited more than two staminate flowers per foraging bout while the smaller bees (*Lasioglossum* sp. and *P. hildebrandti*) visited one and two flowers, respectively per foraging bout (Table 2). Time spent during the foraging bouts was significantly different; $F_{6, 709}$ = 131.364, N = 749, P≤0.001 in 2009 and ($F_{6, 108}$ = 42.104, N = 130, P≤0.001) in 2010. Although, smaller bees such as *Lasioglossum* sp. and *P. hildebrandti* made an average of not more than one visit to staminate flowers during any foraging bout, they took longer to handle these flowers than compared to their bigger bodied counterparts. *A. mellifera* and *Xylocopa* spp. that made more than two to staminate flowers per foraging bout (Table 2).

Due to the lack of reward in the pistillate flowers, pollinators are likely to visit more than one staminate flower during each foraging bout. In fact, only one single visit to pistillate flower was observed by *X. flavorufa* in 2009. The time a pollinator species spent on single staminate flowers during a foraging bout in 2009 were significantly different between species ($F_{6, 303}$ = 19.723, N = 334, P≤0.001). Pistillate flowers occasionally received single visit from *Lasioglossum* sp., *A. mellifera, P. hildebrandti* but these visits were brief as compared to the duration of the visits by the same pollinator species to the staminate flowers. *Lasioglossum* sp. spent averagely 302.7 ± 13.26 s (N = 109) on staminate flowers during a single foraging bout but only 80 s (N = 1) on single pistillate flowers on a foraging bout while *A. mellifera* spent 39 ± 24.09 s (N = 173) on staminate flowers but only a third of that time (11.0 ± 1.0 (2) on pistillate flowers. *Xylocopa* spp. spent the least time on both multiple and single staminate flowers during a foraging bout (Tables 2 and 3). Irrespective of the pollinator species, pistillate flowers were only visited once within any foraging bout. In most cases of few visits to pistillate flowers, a visitor landing on the flower flew away from the

Table 1. Floral visitors observed on the flowers of *M. charantia* and their proportional visits to staminate and pistillate flowers, 2009 and 2010.

Bee family/year	Species name	No. of visitations observed	Frequency (%)	Total no. of staminate flowers visited *	Total no. of pistillate flowers visited
2009					
Apidae	*A. mellifera*	507	46.3	1705	3
	P. hildebrandti	300	27.3	487	3
	X. calens	62	5.7	239	0
	X. flavorufa	53	4.8	211	1
	X. inconstans	34	3.1	138	0
	X. nigrita	25	2.3	102	0
	Amegilla sp.	1	0.1	2	0
	H. gribodoi	1	0.1	1	0
Subtotals		983			
Halictidae	*Lasioglossum* sp.	110	10.1	123	0
Megachilidae	*Megachile* sp.	1	0.1	1	0
	Muscid fly	1	0.1	2	0
Total		1095	(100)	3011	7
2010					
Apidae	*A. mellifera*	281	60.8	447	2
	P. hildebrandti	52	11.3	56	0
	X. calens	4	0.9	17	0
	X. flavorufa	3	0.6	13	0
	X. inconstans	5	1.1	17	0
	X. nigrita	4	0.9	16	0
Subtotal		349			
Halictidae	*Lasioglossum* sp.	113	24.5	117	1
Total		462	(100)	683	3

*Includes also multiple visits to flowers during an observation.

plot after a very short time without visiting any other flowers. But, before flying away, *A. mellifera, Lasioglossum* sp. and *P. hildebrandti* were observed to search for nectar reward from the base of the stigma.

Fruit set and quality in pollination treatments

In both years 2009 and 2010, all bagged flowers (pollinator exclusion) aborted without setting fruits. Fruit set in both years was low for the open pollination treatments, but improved with additional manual pollen deposition on the pistillate flowers (Tables 4 and 5). Hand cross pollination resulted in better fruit set (60% in 2009 (N = 15) and 44% in 2010 (N = 18) as compare

Table 2. Mean number of flower visits of the different flower visitors per foraging bout and duration of multiple visits to staminate flowers of *M. charantia*.

Year/pollinator species	Mean no. of visits to staminate flowers per foraging bout ± S. E		Duration of foraging bout in multiple visits to staminate flowers ± S. E	
	2009	2010	2009	2010
A. mellifera	3.4 ± 0.08^{a} (504)	1.6 ± 0.82^{b} (281)	45.5 ± 1.68^{b} (430)	75.4 ± 7.89^{c} (108)
P. hildebrandti	1.6 ± 0.10^{b} (297)	1.1 ± 0.11^{b} (52)	131.4 ± 2.89^{a} (154)	569.8 ± 33.38^{a} (3)
X. calens	3.8 ± 0.26^{a} (62)	4.3 ± 0.38^{a} (4)	28.6 ± 5.45^{b} (55)	39.8 ± 27.25^{c} (4)
X. flavorufa	3.9 ± 0.25^{a} (52)	4.5 ± 0.46^{a} (3)	33.4 ± 5.45^{b} (46)	37.8 ± 28.73^{c} (3)
X. inconstans	4.0 ± 0.33^{a} (34)	3.5 ± 0.36^{a} (5)	31.3 ± 6.83^{b} (31)	38.0 ± 28.73^{c} (4)
X. nigrita	4.0 ± 0.43^{a}(25)	4.0 ± 0.40^{a} (4)	29.7 ± 9.91^{b} (20)	35.5 ± 28.73^{c} (4)
Lasioglossum sp.	1.1 ± 0.18^{b} (110)	1.0 ± 0.07^{b} (113)	112.7 ± 11.93^{a} (13)	303 ± 27.25^{b} (4)

Means followed by the same letter in superscript are not significantly different based on Student-Newman-Keuls (S-N-K) test at 95% significance level. Numbers in parenthesis are number of observation cases.

Table 3. Mean duration of single visits to staminate and pistillate flowers of *M. charantia*.

Year/pollinator species	Duration of foraging bout in single visits to staminate flowers ± s. e		Duration of foraging bout in single visits to pistillate flowers ± s. e	
	2009	2010	2009	2010
A. mellifera	17.1 ± 6.77^{b} (73)	39.0 ± 24.09^{b} (173)	2.5 ± 15.50 (2)	11.0 ± 1.00 (2)
P. hildebrandti	84.6 ± 4.57^{a} (143)	287.6 ± 19.89^{a} (49)	39.3 ± 13.42 (3)	
X. calens	6.4 ± 22.7^{b} (7)	-	-	-
X. flavorufa	4.9 ± 24.63^{b} (6)	-		
X. inconstans	6.3 ± 31.16^{b} (3)	-		
X. nigrita	5.7 ± 25.44^{b} (5)	-		
Lasioglossum sp.	97.7 ± 5.73^{a} (97)	302.7 ± 13.26^{a} (109)	-	80.0 (1)

Means followed by the same letter in superscript are not significantly different based on Student-Newman-Keuls (S-N-K) test at 95% significance level. Numbers in parenthesis are number of observation cases made.

Table 4. Means yield parameters for the different pollination treatment in *M. charantia* in farmlands of Kakamega forest, Western Kenya, 2009.

Treatments/Yield parameter	OP (n =24)	PA (n = 21)	HCP (n = 15)
Fruit set (%)	38	52	60
Weight of fruit (g) ± S.E	2.9 ± 1.90^{a}	4.3 ± 2.03^{a}	9.7 ± 2.41^{a}
Length of fruit (cm) ± S.E	1.7 ± 1.09^{b}	3.6 ± 1.17^{b}	8.3 ± 1.38^{a}
No. of matured seed/fruit ± S.E	1.0 ± 1.23^{b}	1.7 ± 1.32^{b}	8.5 ± 1.56^{a}
Weight of matured seeds/fruit (g) ± S.E	0.1 ± 0.06^{a}	0.1 ± 0.06^{a}	0.06 ± 0.06^{a}

Means followed by the same letter in superscript within arrow are not significantly different based on Student-Newman-Keuls test at 95% significant level. Number in parentheses represents N value. OP= “Open pollination”, PA = “Pollen augmentation”, HCP = “Hand cross pollination”.

to the open pollination 38% (N = 24) and 14% (N = 37) respectively. A few single visits by *A. mellifera, Lasioglossum* sp. and *P. hildebrandti* were recorded in 2010. Of the 14 single visits recorded for *A. mellifera*, 86% set fruit and 14% were aborted. However, all the single visits by *P. hildebrandti* (N = 2) and *Lasioglossum* sp. (N = 3) were observed to have fruit set.

ANOVA indicated a high significant difference among the pollination treatments. Weight of the fruit was significantly different in 2010 ($F_{2, 85}$ = 7.608, N = 88, P = 0.001 in 2010) but not in 2009 ($F_{2, 57}$ = 2.589, N = 60, P = 0.084). Hand cross pollinated fruits was heavier than other pollination treatments (Tables 4 and 5). The length of the fruits were significantly different ($F_{2, 57}$ = 7.297, N = 60, P<0.01 in 2009 and $F_{2, 85}$ = 11.793, N = 88, P<0.001 in 2010). Longer fruits were recorded in both hand cross

Table 5. Means yield parameters for the different pollination treatment in *M. charantia* in farmlands of Kakamega Forest, Western Kenya, 2010.

Treatment/Yield parameter	OP (n =37)	PA (n =33)	HCP (n = 18)
Fruit set (%)	14	61	44
Weight of fruit (g) ± S.E	0.6 ± 0.60[b]	3.9 ± 0.63[a]	3.0 ± 0.86[a]
Length of fruit (cm) ± S.E	0.8 ± 0.77[b]	6.2 ± 0.81[a]	3.8 ± 1.1[a]
No. of mature seed/fruit ± S.E	0.3 ± 0.77[b]	4.3 ± 0.82[a]	4.3 ± 1.2[a]
Weight of mature seeds/fruit (g) ± S.E	0.04 ± 0.09[b]	0.60 ± 0.11[a]	0.4 ± 0.14[a]

Means followed by the same letter in superscript within arrow are not significantly different based on Student-Newman-Keuls test at 95% significant level. Number in parentheses represents N value. OP = "Open pollination", PA = "Pollen augmentation", HCP = "Hand cross pollination".

and in pollen augmentation pollination treatments (Tables 4 and 5). The number of mature seeds per fruit were found to be significantly different between pollination treatments ($F_{2, 57}$ = 7.891, N = 60, P = 0.001 in 2009 and $F_{2, 88}$ = 7.835, N = 88, P<0.001 in 2010). The weight of mature seeds were not significantly different among pollination treatments in 2009 ($F_{2, 57}$ = 0.473, N = 60, P>0.05) but was significantly different in 2010 ($F_{2, 85}$ = 7.264, N = 88, P = 0.001). Both hand and supplemental hand cross pollination resulted in higher fruit weight, length and mature seed numbers ad compared to natural pollination. Fruit weight, length, number of mature seeds and weight of dry seeds were not significantly different (P>0.05) amongst the single visits by *A. mellifera*, *P. hildebrandti* and *Lasioglossum* sp.

DISCUSSION AND CONCLUSIONS

Results indicated differences in floral display and flowering time between the staminate and pistillate flowers of *M. charantia*. The long period of overlap of flowering of both flower sexes plus the numerous staminate flowers enhanced the pollen flow and the overall pollination of the crop. *Lasioglossum* sp., *A. mellifera, P. hildebrandti* were considered potential pollinators of *M. charantia* in Kakamega, Western Kenya. These flower visitors clearly discriminated against the rewardless pistillate flowers. The proportion of pistillate flowers that received visitations from flower visitors was low representing 1% of all the 3693 visitations by floral visitors. *Lasioglossum* sp., *A. mellifera, P. hildebrandti* visited few flowers per foraging bout and the visits were very brief compared to the larger *Xylocopa* spp. who visited more flowers per foraging bout. It is possible that once the flower visitors experienced the rewardless pistillate flowers, second time foragers avoided these flowers in their subsequent visits all together and that pollination of this crop was purely by deceit. Reproductive success of *M. charantia* was pollen limited. This can be attributed to the high discrimination against the pistillate flowers that would have otherwise developed into fruit, that is, in case, it received visits from pollen loaded flower visitors. Flowers receiving hand cross pollination resulted in 20% higher fruit in 2009 and 30% in 2010 while supplemental hand cross pollination increased fruit set by 14% in 2009 and 40% in 2010 when compared to open pollinated (control) flowers. Supplemental hand cross pollination did not result into increased fruit set when compared to hand cross pollination. Given the high discrimination against the pistillate flowers, it is possible that the treated pistillate flowers in this treatment did not receive any additional visits from flower visitors or the amount of pollen that was deposited on the stigma by hand was definitely enough for highest possible fruit set. Also, since the amount of pollen dusted onto the stigma was not counted, it is possible that too much pollen was deposited leading to stigma pollen clogging and reduced fruit and seed set. On the other hand, low fruit set in the open pollination treatment for 2010 compared to 2009 was attributed to the differences in pollen flow in both years. For instance, there was a high ratio of pistillate to staminate flowers (1:430 in 2009 compared to 1:227 in 2010). This meant that in 2009, there was more pollen available to flower visitors during foraging and that enough was deposited on the pistillate flowers hence better fruit set. Fruit set differences in hand cross pollination in 2009 (60%, N = 15) and 2010 (44%, N = 18) can be attributed to high rainfalls experienced in Kakamega during (the long rains) the growth period of the test crop in 2010 than in 2009 (MEMR, 2009; 2010). *M. charantia* is reported to perform better under well drained soils (Joseph, 2005; Behera *et al.*, 2010). The high rains might have caused waterlogged soil conditions and therefore, reduced the growth and performance of the crops.

The absence of nectar in the pistillate flowers due to either lack of or reduced nectaries have been recorded in other Cucurbitaceae, for example, *Momordica* spp., *Lagenaria* spp., *Luffa* spp., (Bahadur et al., 1986), *E. elaterium* (Fahn and Shimony, 2001) and *M. charantia* (Lenzi et al., 2005). High staminate flower mimicry by the pistillate flowers was evident in this study. Mimicry of staminate flowers by the rewardless pistillate flowers have been reported in a number of plant species including, *C. papaya* (Baker, 1976), *E. elaterium* (Dukas,

1987), and several *Begonia* species (Ågren and Schemske, 1991; Schemske et al., 1996). Recently, mimicry by the pistillate in *M. charantia* was observed by Lenzi et al. (2005) in Brazil and Deyto and Cervancia (2009) in Philippines. The high numbers of staminate flowers relative to pistillate flowers have been reported on *M. charantia* in India (Joseph, 2005) and Brazil (Deyto and Cervancia, 2009). It is believed that the higher number of the staminate flowers enhances the chance of effective pollination, resulting in high fruit and seed set; current research on yield improvement of this crop has focused on increasing the number of pistillate flowers (Behera, 2004). The results in this study indicated that, numerous staminate flowers ensures high pollen flow and enough pollen was collected and dusted on the foragers' body such that single "chance" visits have enough pollen deposited on the stigma for fruit set. However, there is need to evaluate the optimal ratio of pistillate to staminate flowers for maximum fruit production.

A. melifera, *P. hildebrandti* and *Lasioglossum* sp. as well as, *Xylocopa* spp. have been recorded with other plant species in the Cucurbitaceae family and in particular *Momordica* species. Binkenstein (2009) recorded *A. mellifera*, *Lasioglossum* sp., *Hypotrigona gribodoi* among other bee species visiting flowers of *M. foetida* in the Kakamega farmland, Kenya. Sommeijer et al. (1983) recorded *A. mellifera* and *Tetragona* spp. visiting flowers of *M. charantia* in Trinidad. Behera (2004) noted *A. florea, A. cerana*, and *A. dorsata* as important pollinator of *M. charantia* in India. Deyto and Cervancia (2009) recorded *Xylocopa* sp., *Halictus* sp. *and Trigona* sp. amongst others as frequent visitors to *M. charantia* in Philippines. However, the current observed pollinators contradict those of Lenzi et al. (2005), who indicated *Diabrotica speciosa* (Coleoptera, Chrysomelidae) to be the main pollinator of *M. charantia* in Brazil.

Strong discrimination between staminate and the rewardless pistillate flowers by all the pollinators observed in this study concurs with other studies that have indicated that rewardless pistillate flowers in both monoecious and dioecious plants received less or no visits by pollinators when compared to the staminate flowers (Ågren et al., 1986; Bierzychudek, 1987; Dukas, 1987; Ågren and Schemske, 1991; Schemske *et al.*, 1996; Le Corff *et al.*, 1998; Kawagoe and Suzuki, 2002; Rust et al., 2003). In this study, the time spent on flowers was considerably shorter for the bigger sized bee species, for example, *Xylocopa* spp. as compared to the small bodied bee species; *A. mellifera*, *P. hildebrandti* and *Lasioglossum* sp. Larger bees visited more flowers in the afternoon, while smaller bees did not change their flower visiting behaviour during the day. For larger *Xylocopa* spp., the number of visited flowers increased significantly in the afternoon possibly due to smaller nectar amounts in the afternoon. Hoehn et al. (2010) found body size to influence the foraging behaviour of pollinator. He indicated that bigger bodied bees like the *Xylocopa* spp. visited more flowers though for a shorter time when compared to smaller bees. Since staminate and pistillate flowers are opened only for one day, the discriminatory ability of these pollinators shown by the low frequency of visits to the relatively low number of female flowers definitely reduces the female reproductive success of *M. charantia*.

Fruit set results indicated that *M. charantia* is a pollinator limited crop and supplemental pollination is necessary for the production of marketable fruits. The poor fruit set in this study agrees with results by Mishra and Sahoo (1983) who reported fruit set of 22% under natural pollination conditions. On the contrary, results by Deyto and Cervancia (2009) showed high fruit set in natural pollination (78%) and there was no significant difference with hand pollinated flowers (80%). Although, not mentioned in the study, the high fruit set in natural population by Deyto and Cervancia (2009) could have been as a result of high abundance of foragers in the test plot. Under normal conditions, it is assumed that every flower was visited more than once and probably by many different bee species, as such, they will have a better fruit and seed set, but this was not the case for *M. charantia* in the study area. Poor yields were consistent with observations on low visitation rates to the pistillate flowers. Low visitation rates to pistillate flowers combined with the low female to male flowers ratios observed contributed highly to the poor fruit set. In agricultural production, where higher fruit set, bigger fruits with more uniform shapes are desired, two options are possible to increase the fruit set of *M .charantia*, (1) the introduction of managed (stingless) pollinator populations for example, *A. mellifera*, *P. hildebrandti*, (2) farmers must resort to hand pollination to produce any marketable fruits. Indeed, other authors have recommended hand pollination for commercial crop and seed production (Behera et al., 2010; Joseph, 2005; Devadas and Ramadas, 1993; Mishra and Sahoo, 1983). Devadas and Ramadas (1993) calculated that 29 man-hours were needed to produce 1 kg of commercial seeds. Hand pollination requires labour resource input and is an added cost to the already resource poor farmers. On the other hand, hand pollination may not necessarily result into better fruit set because of pollen clogging due to too much pollen deposited on the stigma. *A. mellifera, Lasioglossum* sp., and *P. hildebrandti*, showed a high efficiency of pollen transfer as depicted by the high fruit set in the single visit when compared to other pollination treatments. It was also evident that native bee communities were unable to offer sustainable pollination service. Therefore, pollination augmentation or managed crop pollination should form core inputs considered in the production of this crop. Increasing the density of pollinator populations by introduction of hives into the flowering crop of *M. charantia* will result into competition for nectar and pollen forcing bees to visit even the female flowers. *Lasioglossum* sp. recorded visiting *M. charantia*

in this study, nests in soil and other pithy stems of herbs and shrubs (Gikungu, 2006). Farming practises such as maintaining of diverse hedges around farmland landscapes increases the abundance of such pithy stems thereby, enhancing the nesting sites for these bees. *P. hildebrandti* nests in occupied termite mounds at the core of the mound (Michener, 2000). However, termite mounds as well as, the termites themselves are in most cases are cleared off from the farmlands using pesticides. Such practises may accelerate the decline in abundance of these pollinators thereby, reducing crop fruit and seed set.

Meliponiculture provides new emerging frontiers for alternative pollinators for crop production and income generation for the farmers. Observation of *P. hildebrandti* visiting flowers of *M. charantia* is in particular of much interest and highlights the potential of stingless bees for crop pollination of commercially important crops (Heard, 1999; Slaa et al., 2006). More research is however, needed to evaluate if the introduction of honey/stingless bee hives into the crop results into less pistillate flower discrimination and higher fruit yields. Further research on the potential of stingless bees and solitary bees as manageable crop pollinators will be of interest.

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