

Diversity and efficiency of wild pollinators of watermelon (*Citrullus lanatus* (Thunb.) Mansf.) at Yatta (Kenya)

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Abstract

Citrullus lanatus (Thunb.) Mansf. (Watermelon) is an important crop plant in Kenya. Being monoecious, watermelon is entirely dependent upon pollination services usually by insects for production. Although the centre of origin for this plant is thought to be tropical Africa, essentially not much has been studied of its pollination requirements in this region. The current study investigated the identity of the wild pollinators of watermelon, their behaviour and relative pollination efficiencies at Yatta, a farm near Thika (Eastern Province). The main pollinator for this crop was found to be the honey bee, *Apis mellifera* but three wild species of *Lasioglossum* were found as important pollinators. These wild bees have a significantly higher ($P < 0.0001$) pollen deposition on stigmas of watermelon than honeybees. One of the *Lasioglossum* (*Ctenonomia*) sp. 4 deposited on average three times as much pollen as the honeybee. At about the time of stigmatic receptivity, the number of visits by this species to female flowers increases until it equals visits to male flowers irrespective of number of flowers per plot. This behavioural pattern coupled with the high pollen deposition potential makes *Lasioglossum* (*Ctenonomia*) sp. 4 a superior candidate as an alternatively managed pollinator for watermelon. Knowing that visitation occurs mostly in the morning, and that flowers last only for one day, spraying can be done in the later hours of the day when the pollinators have virtually stopped foraging on the flowers. In view of the reported pollinator decline globally, the wild pollinator species reported in this study warrant further investigation on their nesting biology and potential for domestication.

Key words: Native pollinators, watermelon, visitation patterns, pollen deposition

Introduction

The global research, conservation and agricultural communities are increasingly recognising that pollinator diversity and effectiveness is critical to the health of agricultural ecosystems. The contribution made to plants' reproductive success by pollination is determined in part by the pollinators foraging behaviour. Many insect visitors forage on the floral resources presented to them by the plant but only a few are good pollinators. Information on actual or potential role of individual pollinator species is necessary to compare the potential value of different pollinator species as candidates for introduction as managed pollinators (Corbet *et al.*, 1991). The most efficient pollinator will carry and deposit plenty of pollen on stigmas as it moves from flower to flower (Kearns and Inouye, 1993).

The consequences of pollinators visiting a plant can be expressed by either direct or indirect measure of pollinator effectiveness. The indirect methods include visitation rates and time of visitation while pollen load deposition by a single visit to virgin stigmas is a direct measure among others (Spears, 1983; Inouye and Pyke, 1988; Stubbs and Drummond, 1999; Dag and Kammer, 2001).

Pollinator effectiveness will of course be related to the timing of visits. If an insect arrives at a flower to take nectar before pollen is presented, or forages after pollen is shed or stigmas have ceased to be receptive, they will not be contributing to reproductive success.

Thus for a pollinator dependent plant such as the watermelon, the temporal pattern of pollinator visitation becomes significant in determining important or effective pollinators. Although the visitation patterns of honeybee on watermelon have been studied (Njoroge *et al.*, 2004; Gikungu, 2006; Kasina, 2007; Karanja, 2010), it is important to compare these visitation rates with those of other important indigenous pollinators as an indicator of relative importance.

For crops that are dependent on insect vectors such as pollinators, managed honey bee colonies are often used to provide pollination services in developed countries or those with a strong apiculture development. In developing countries such as Kenya, however, where most horticultural crops, until recently, have been grown in biodiverse smallholder systems, there is no tradition of providing pollination services, and pollination deficits have not been recognised as a constraint in productivity. This changing scenario in horticultural sector is intensifying and expanding, resulting in greater uniformity in crop fields, greater fragmentation of natural habitats, and greater use of agrochemicals. The challenge, for agroecosystems under development in Kenya, is to identify means of conserving and managing natural pollination services, as a much needed basis for pollination needs. Supplementation by managed honeybee populations may effectively be combined with the natural system, with both providing an effective insurance against risks of disease, agricultural chemicals, and inclement weather impacts on diverse pollinator species.

Tropical Africa is endowed with great diversity of both fauna and flora. It has been estimated that East Africa has about 240 species of bees in 7 families but the diversity and taxonomy is poorly known (Gikungu, 2002). *Ctenonomia*, for example is a major group of *Lasioglossium* bees in Africa. Although the group has many species, it is poorly known in Africa (Mischener, 2000). Systematic characterisation of bees acting as pollinators of plants critical for human livelihoods and ecological sustainability is not only relevant but urgent.

In Kenya, studies in pollination biology have been focused mainly on the ecology and taxonomy of the honeybees (Kigatiira, 1974, 1976, 1984; Mbaya, 1981; Kahenya and Gathuru, 1985; Ng'ethe, 1985). Recently some studies have been done on pollination biology targeting both natural and agroecosystems in Kenya (Gemmill and Ochieng, 1999; Gikungu, 2002; Siro, 2004; Morimoto *et al.*, 2004; Njoroge *et al.*, 2004; Gikungu, 2006; Kasina, 2007; Karanja, 2010). There is need to document more extensively important plant-pollinator interactions so that conservation measures can be put in place.

The importance of pollination in watermelon: It is believed that *Citrullus lanatus* (watermelon) is native of tropical Africa (Cobley, 1965; Masefield *et al.*, 1969; Kirkbrid, 1993). Its cultivation began in ancient Egypt and Sudan then spread from there to other countries via the Mediterranean, Near East and Asia. From immuno-chemical data the crop is thought to have originated from its semi cultivated variety *Cordophanus* found in Sudan (Fursa and Gavrilyuk, 1990). The long association of *Citrullus*, and many other cucurbits crops with human settlements has indicated that there is no clear demarcation between wild relatives and crop varieties (Heiser, 1969).

Watermelon is now cultivated in many parts of the tropics (Cobley, 1965; Masefield *et al.*, 1969). The fruit is an important source of vitamins, minerals, carotenes and proteins. It is also a rich source of natural lycopene, a carotenoid of great importance because of its antioxidant property (Denton, 2004). Highly prized oil is now being extracted from seeds of watermelon. This oil is used for cooking, cosmetic purposes and is of importance in pharmaceutical industry (Denton, 2004).

Global production of watermelon has increased from 30 million tons (2.1 million ha.) in 1992 to 81 million tons (3.2 million ha.) in 2002 (Denton, 2004). In Kenya, the growing of watermelon is increasing, especially in the arid and semi arid areas under irrigation.

This study aimed at investigating the time of the day when watermelon has most visitations by the main pollinators so as to furnish farmers and pollination managers with data that can be applied in integrated pest management for pollinator safety and conservation. Daily visitation patterns are also useful in assessing the effective pollinators for a given plant and region. Further, an investigation was sought to establish the full cast of pollinators of watermelon at Yatta (Eastern Province, Kenya) and the efficacies of the four main pollinators with regard to pollen deposition on virgin stigmas.

Materials and methods

Growing conditions of the crop: Plants were sown in nursery beds at Yatta National Youth Service farm near Thika and transplanted on ridges with planting distance of 3×2 m. A total of 1000 plants were sown in early December so that flowering and fruiting could coincide with the dry months of January and February. The plants were top-dressed with calcium ammonium nitrate (C.A.N.) fertiliser when the plants began to spread and again just before flowering (IRACC, 2000). Drip irrigation was used every second day.

Observation on anthesis: The flowers were observed to indicate time of opening and closing for both male and female flowers. To estimate the approximate time of the day when anthers are dehisced, the anthers were stroked gently with a small paintbrush. At every stroke the brush was examined for the presence of any pollen grains adhering on it. From 6.30 a.m. the anthers were stroked every 30 minutes until some pollen grains were observed on the paintbrush. At the same time the stigmas were observed for receptivity every 30 minutes until a glassy stigmatic secretion was observed (Owens, 1992).

Diversity in insect visitors: In documentation of flower visitors special attention was paid to discriminate between pollinators and mere floral visitors. Those insects that evidently carried watermelon pollen and had contacts with anthers and stigmas were recorded as pollinators. Representatives of pollinators were captured and killed in a jar using ethyl acetate fumes. Voucher specimens were prepared, insects identified and then preserved in insect boxes and deposited at Jomo Kenyatta University, Botany Department Laboratories.

Pollinator behaviour: Initially observations for any visitors to the flowers began at 6.30 a.m. but it was noted that no visitor arrived before 7.30 a.m. Consequently, recording of insect foraging behaviour and visitation patterns began at about 7.30 a.m. and ended at about 4.00 p.m. when all the flowers closed. Observations were standardized by counting the number of flowers on 1 m² plots of the flowering crop and the number and types of pollinators coming to the flowers recorded over 10-minute periods of time (Inouye and Pyke, 1988; Motten, 1986; Wolf *et al.*, 1999).

Measurement of pollinator efficiency: The number of pollen grains deposited on the stigma by a single pollinator visit is usually used to estimate relative pollinator efficiency (Yeboah and Woodell, 1987). Female flowers were bagged while in bud stage to prevent any contact of stigmas with pollinators. When the flowers were open, receptive stigmas were held in a long rod and presented to the pollinators. The pollinator was followed to ensure it has consistently visited male watermelon flowers before the virgin female flowers were presented (Kearns and Inouye, 1993; Stanghellini *et al.*, 2002). When the targeted pollinator made one visit, the stigmas were picked and crushed on a microscope slide and kept in a cool box in the field. The crushed stigmas were then observed in the laboratory under a microscope and the number of pollen grains deposited counted. Ten stigmas were presented to each of the four main pollinators of watermelon. The mean number of pollen grains deposited was calculated and compared between the four pollinators by t-test. Genstart software was used for all the data analysis.

Results and discussion

Anthesis in watermelon: The male flowers began to open at about 7.15 a.m. and began to close at 1 p.m. On the other hand the female flowers began to open at about 7.20 a.m. and closed much earlier at about 12 p.m. Sometimes when the temperatures were high some female flowers begin to close at 11.30 a.m. The anthers dehisced from 8.30 a.m. while the stigmas became receptive from 10.30 a.m.

Pollinator diversity in watermelon: The main pollinator of watermelon in this region was found to be the honeybee (*Apis mellifera* L.). Three wild bee species, *Lasioglossum (Ctenonomia) scope*, *Lasioglossum (Ctenonomia) sp. 4* and *Lasioglossum (Ipomalictus) nairobiense* were observed to be regular pollinators of watermelon though not as regular as the honeybee. The first two, make use of ground conditions below the watermelon crop to nest.

Other insects however, were also flower visitors of this crop. The following may not be important pollinators of watermelon in the study area but benefited by obtaining nectar and/or pollen: *Danaus chrysippus*, *Eurema brigitta*, *Neocoenyra gregorii*, *Junonia hierta*, *Aphthona marshalii*, *Leptaulaca fissicollis*, *Xylocopa lataritia*, *Anthophora bipartite*, *Braunsapis sp.*, *Nomia sp. 1*, *Macrogalea sp. 1*, *Trinomia cirrita*, *Lipotriches hylaeoides*, *Chrysomya sp.*, *Cosmina sp.* and *Phytomyia sp.*

Although previous studies on watermelon in Kenya (Njoroge *et al.*, 2004) and in other regions (Rao and Suryanarayana, 1988; Sanford, 1992; Hodges and Baxendale, 1995; Stanghellini *et al.*, 1998) have shown that honey bee is the main pollinator

of watermelon, this study however, identified three wild *Lasioglossum* species, which have not been reported before, as pollinators of watermelon. In the past, most efforts to conserve and sustainably use pollinators for agricultural production have been focused on the honeybee (Cane, 1997). Wild pollinators however are an important reservoir of pollination capacity with potential for application in agricultural production. The wild bees identified in this study are a valuable reservoir, which can be considered for management in improving watermelon production in the area.

Daily pollinator visitation patterns in watermelon: The daily visitation patterns of the three most common bee species that evidently had contact with anthers and stigmas of watermelon and were seen to have substantial pollen as they left the male flowers (*Lasioglossum (Ctenonomia) scope*, *Ctenonomia sp. 4* and *Lasioglossum (Ipomalictus) nairobiense*) were compared with honey bee patterns (Fig. 1a-d). The honeybee was the first visitor at about 7.30 a.m. followed by *Lasioglossum (Ctenonomia) scope* and *Ctenonomia sp. 4* at about 7.40 a.m. The *Lasioglossum (Ipomalictus) nairobiense* arrived only after 8.30 a.m., This visitor was rather more sporadic than the others and seemed to avoid the female flowers but had evident watermelon pollen on its body. All the four species made more visits to male flowers than female flowers, except *Ctenonomia sp. 4* (Fig. 1c), which at about 11.30 a.m. made equal number of visits in male and female flowers irrespective of the number of flowers of each sex in the 1 m² plots.

In the case of honeybees, visits were few before 8.00 a.m. but there was increase in number of visits to both male and female flowers gaining a peak at about 10.40 a.m. (Fig. 1a). The highest

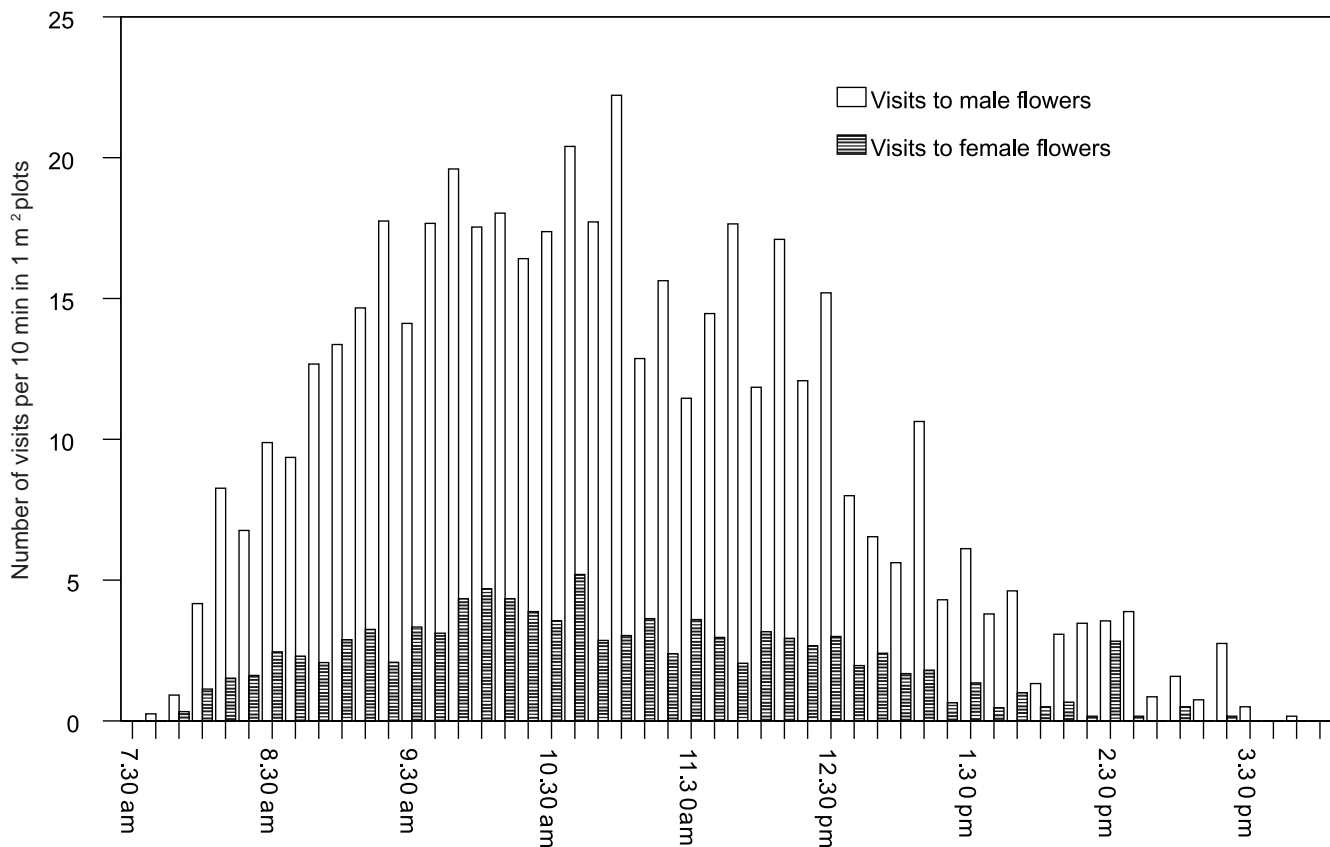


Fig. 1a. Honeybee daily visitation rates on watermelon flowers every 10 min in 1 m² plots

mean visits to male flowers were observed at about 11.00 a.m. while in female flowers this was observed to be at about 10.40 a.m. After 12 p.m. the visits decreased gradually but more so in female flowers as most of them began to close.

Lasioglossum (Ctenonomia) scobe began appearing on watermelon flowers at about 7.40 a.m. (Fig.1b) visiting male flowers only up to 8.30 a.m. when it began to visit female flowers. This pollinator

visited the male flowers up to 12 p.m. but the female flowers up to 11.30 a.m. Most visits to female flowers were made between 10.00 a.m. and 10.40 a.m., a time when the female flowers had become receptive.

Ctenonomia sp. 4 began foraging on watermelon male flowers at about 7.50 a.m. with maximum visitation occurring at about 8.30 a.m. (Fig.1c). This species foraged on female flowers between

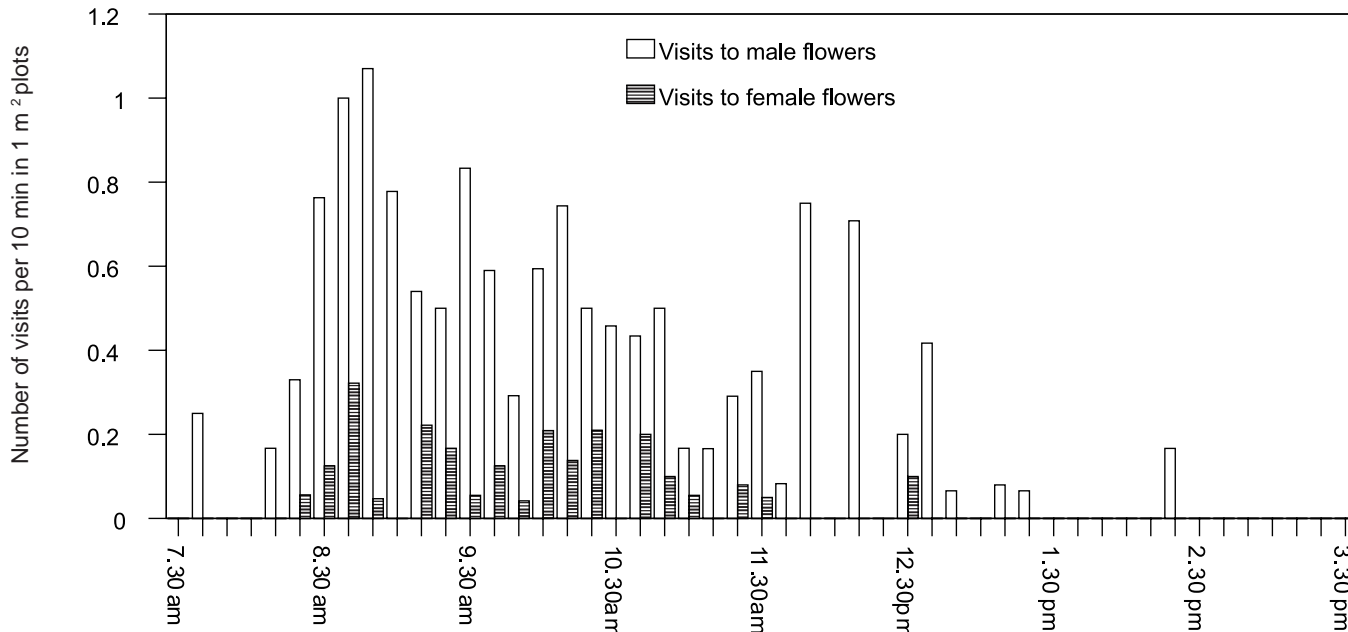


Fig. 1b. *Lasioglossum (Ctenonomia) scobe* daily visitation rates on watermelon flowers every 10 min in 1 m² plots

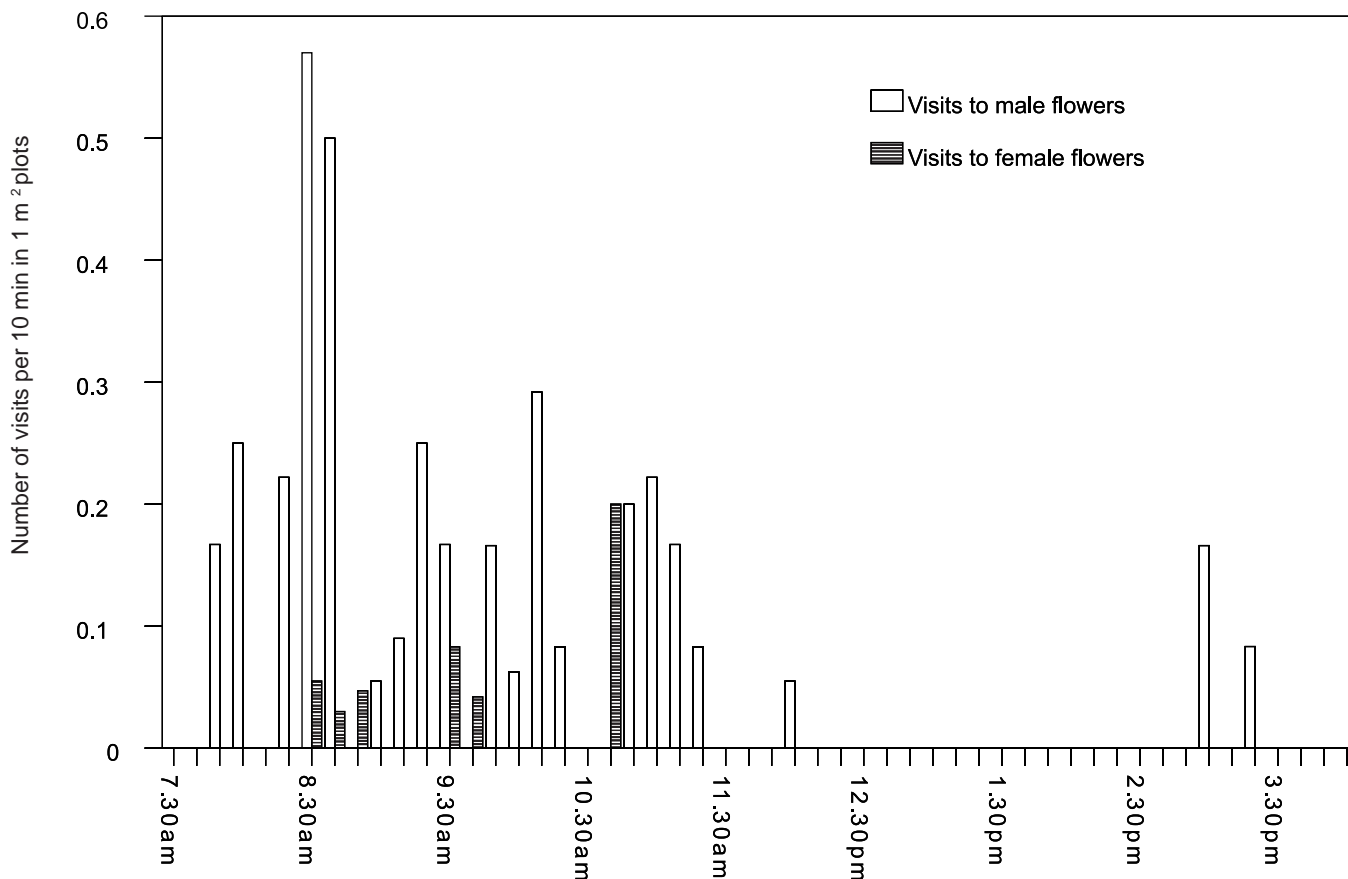


Fig. 1c. *Lasioglossum* sp. (*Ctenonomia*) sp. 4 diurnal visitation rates on watermelon flowers every 10 min in 1 m² plots

8.30 a.m. and 10.40 a.m. Maximum visitations to female flowers occurred at 10.40 a.m. when the female flowers were receptive. *Lasioglossum (Ipomalictus) nairobiense* appeared on the crop at about 8.40 a.m. (Fig. 1d). It regularly visited male flowers between 8.40 a.m. and 9.20 a.m., then again at about 12.00 p.m. and did not come back to the crop for the rest of the day.

It was observed that only a few honeybees, *Lasioglossum (Ctenonomia) scobe* and *Ctenonomia* sp. 4 made occasional appearances after 1.00 p.m. mostly to male flowers. No pollination would be possible after this time as the female flowers began to close at about 12.00 p.m. Where bees visits were observed in female flowers after 12.00 p.m., they were forcing their way into already closing female flowers.

Daily visitation patterns to male and female flowers are particularly useful in unisexual plants as they help establish if the main pollinators are discriminating either of the sex of flowers during foraging. The absolute number of visits to female and male flowers in this study showed that the latter were visited more than the former. But when the total visits were compared with the proportion of male and female flowers in 1 m² of the crop the bees tended to show a preference for female flowers. These results are contrary to those obtained in a study of the *Antennaria parvifolia* Nutt. (Asteraceae) where pollinators have been shown to increase the cost of sex by avoiding female flowers (Bierzychudek, 1987). There are several plant families where studies have shown pollinators discriminate against female flowers. These include Saliaceae (van der Werf, 1983), Caricaceae (Baker, 1976) and Sapindaceae (Bawa, 1977) to name only a few.

Such observations have led to the general conclusion that visits to female flowers may be made only by mistake. It has therefore been predicted that discrimination against female flowers is a common

phenomenon among dioecious and monoecious species. In the case of watermelon (monoecious) the three species of pollinators: honeybee, *Lasioglossum (Ctenonomia) scobe* and *Ctenonomia* sp.4 are all found to decrease the cost of sex by seeking out for female flowers in their foraging. The current study did not confirm the general prediction that pollinators of unisexual plants increase the cost of sex. Since the family Cucurbitaceae in Kenya has many unisexual species, pollinator visitation patterns should be investigated in the other species including wild species.

Pollinator daily visitation patterns have been found to have implications on the pollinators' effectiveness (Spears, 1983; Stanhellini *et al.*, 2002). In this study, the honeybee was found to be the most abundant and regular pollinator to watermelon flowers. The maximum visitation of *Lasioglossum (Ctenonomia) scobe* and *Ctenonomia* sp.4 to female flowers coincided with stigmatic receptivity while in male flowers it corresponded to time of anther dehiscence in this crop. This synchronisation makes them effective pollinators as they first make visits to male flowers, collecting loads of pollen and then make visits to female flowers facilitating dislodgement of the pollen on the already receptive stigmas.

The daily visitation patterns also find application in pest management. A primary concern for beekeepers, when involved in contracting their bees for pollination, is the use of pesticides by the growers (Sanford, 1992). From these results, it is recommended that pesticides be applied when these bees have left the flowers for example late in the evening. Since the flowers for that day will have closed and new flowers will emerge the following morning, the bees stand some chance of escaping poisoning. The fresh flowers and nectar produced in the following morning will have less direct contact with the pesticide sprayed the previous day, than the flowers sprayed in the morning when they are open. Applying

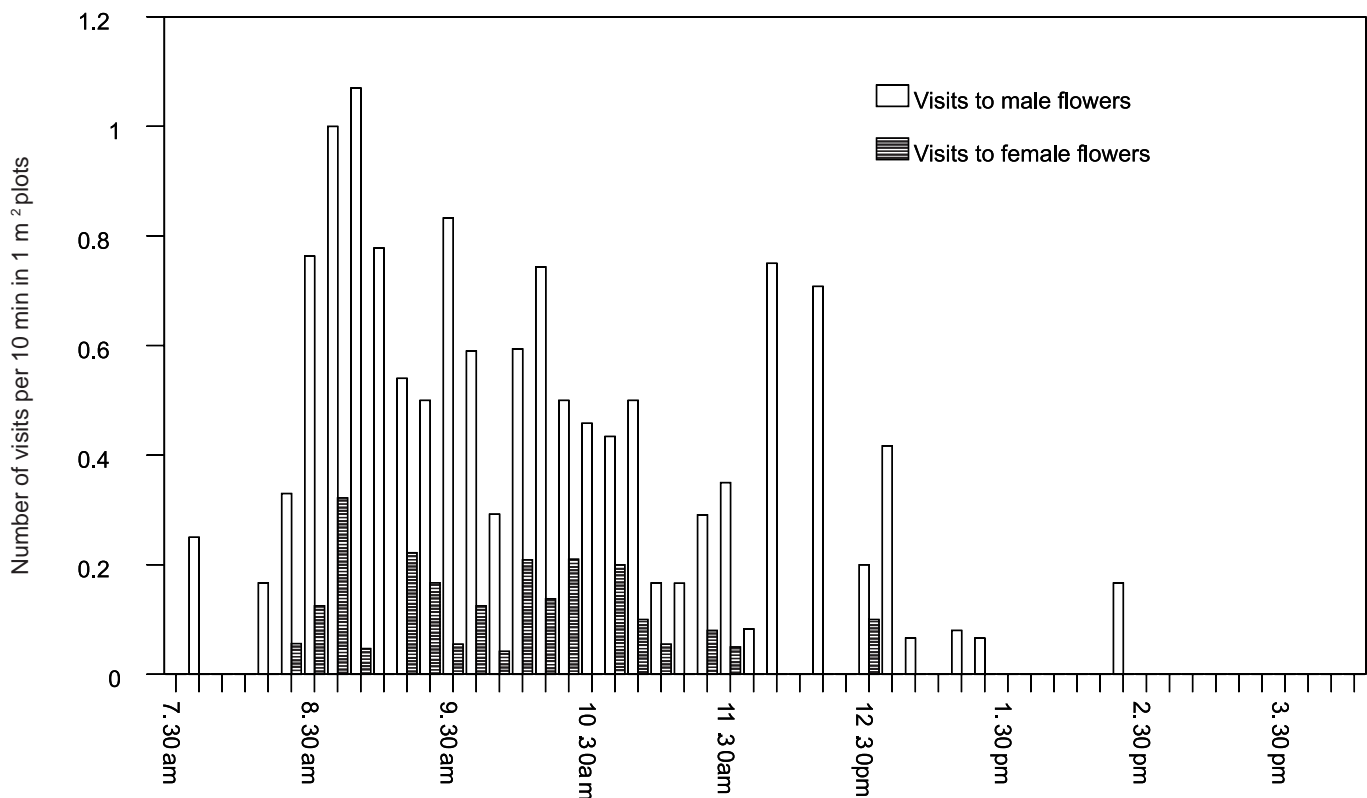


Fig. 1d. *Lasioglossum (Ipomalictus) nairobiense* diurnal visitation rates on watermelon flowers every 10 min in 1 m² plots

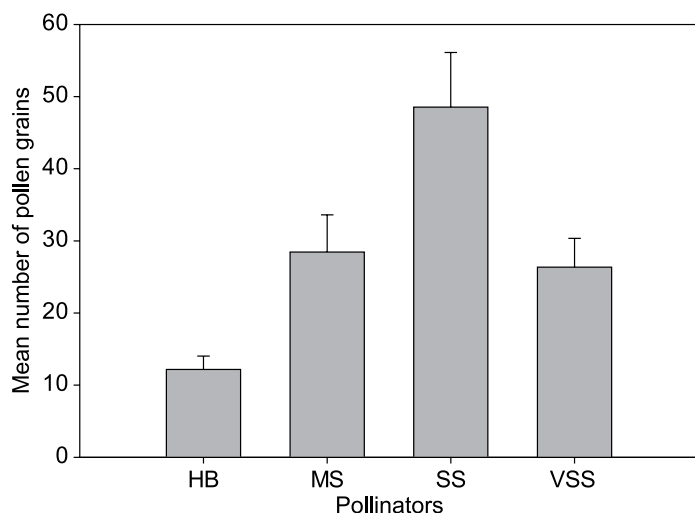


Fig. 2. Comparison of pollen deposits between honeybee and the three wild pollinators of watermelon. (HB= honeybee; MS= *Lasioglossum (Ipomalictus) nairobiense*; SS= *Lasioglossum (Ctenonomia) sp 4*; VSS= *Lasioglossum (Ctenonomia) scobe*).

pesticides in the latter parts of the day will also be important in ensuring pollen germination as some fungicides have been found to reduce *in vitro* pollen germination and the germ tube elongation (Abbot *et al.*, 1991).

Pollinator efficiency: Pollen load analysis revealed that the four insect species have significantly different capacity for depositing pollen ($P < 0.0001$). The highest potential for pollen deposition was found in *Ctenonomia sp. 4* followed by *Lasioglossum (Ipomalictus) nairobiense* and *Lasioglossum (Ctenonomia) scobe* while the honeybee had the least (Fig. 2). Consequently, the three species *Lasioglossum (Ctenonomia) scobe*, *Ctenonomia sp. 4* and *Lasioglossum (Ipomalictus) nairobiense* showed higher pollination efficiency than the abundant honeybee.

This study revealed for the first time in this region the pollinators for watermelon superior to honeybees. Although bees have been pollinating flowering plants for more than 100 million years, only about ten out of the available 20,000 non-parasitic bee species have been managed for agricultural pollination (Cane, 1997). Loss of feral and managed bees (particularly honeybees) has become a limiting factor in pollination of many bee-pollinated crops (Corbet *et al.*, 1991; Stanghellini *et al.*, 1998) leading to the current search in different parts of the world for alternative agricultural pollinators. The current study provides biologists and agriculturists with important watermelon wild pollinators that can be targeted for development and management as alternative pollinators considering their higher pollination efficiencies.

These results support the hypothesis that local pollinators are important in the pollination of watermelon crop that has been bred from the indigenous African *Citrullus lanatus*. Other than the popular honeybees, wild *Lasioglossum* bee species were identified to be important pollinators with a unique relationship with the crop as some of them nest in the soils where watermelon is growing. These bees also forage the male flowers soon after the anthers dehiscence and the female flowers soon after they have become receptive. The indigenous pollinators of a crop are thought to be significant especially in continents where the crop's close relatives are found (Cane, 1997). Since watermelon has its origin in Tropical Africa where wild relatives are found (Cobley, 1965; Kirkbride, 1993; Nayar and More, 1998) these indigenous

pollinators and the pollination biology of wild watermelon as well as other wild cucurbits need to be studied further. Conservation status of these wild pollinators needs to be assessed so that strategies can be worked out to ensure their continued survival and hence more benefits to watermelon production and other plants in the ecosystem.

Limited information on the importance of wild pollinators and their interaction with crops in the past has been a hindrance to the development of appropriate strategies for pollinator conservation and sustainable use. This study will significantly contribute to the understanding of the interaction between watermelon and the diversity of pollinators particularly the wild pollinators. Data from this study will contribute to the local and global understanding of ecosystem service that pollinators provide. This is especially an important key since there is now an established International Pollinator Initiative (IPI), which is a centralised entity to facilitate information exchange on pollinator conservation and sustainable use.

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