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ORIGINAL ARTICLE

Enhancement of fruit quality in *Capsicum annum* through pollination by *Hypotrigona gribodoi* in Kakamega, Western Kenya

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Abstract

A study was carried out in Kakamega forest, in the western region of Kenya, to evaluate the effectiveness of the stingless bee *Hypotrigona gribodoi* (Magretti, 1884) on the pollination of green pepper. Three treatments were applied and consisted of self-pollination, pollination by feral pollinators in the open field and pollination by *H. gribodoi* in a net cage. The differences in fruit yield and seed quality were compared among treatments. Flowers pollinated by *H. gribodoi* produced the heaviest fruits with the highest seed numbers followed by feral pollinators and lastly self-pollinated flowers. Moreover, seeds were significantly bigger in size in fruits resulting from flowers pollinated by *H. gribodoi* compared to fruits obtained from self-pollinated flowers or flowers pollinated by feral insects. We, therefore, conclude that *H. gribodoi* is an efficient pollinator of green pepper in the tropical region of East Africa.

Key words: Hymenoptera, Meliponini, pollination, Solanaceae, yield enhancement.

INTRODUCTION

The green pepper, *Capsicum annum* Linné, 1753 (Solanaceae) originated from Central America and is currently cultivated worldwide (Cruz *et al.* 2005). Flowers of green pepper self pollinate in the absence of pollinators (autogamy) to produce fruits with seeds (Pesson & Louveaux 1984) and flower opening occurs at sunrise (Cruz 2009). Pollination of *Capsicum annum* flowers by some specific insects have been shown to have a positive effect by enhancing fruit quality and seed set (Pesson & Louveaux 1984). In Kakamega, a town in the western region of Kenya, green pepper is one of the main spicy crops cultivated by rural farmers, for their own consumption and for sale in the local market (Kasina *et al.* 2009). However, the use of a specific pollinator in green pepper farming in Kakamega is non-existent and the crop relies entirely on feral pollinators (feral bees and other biotic pollinators) supported by nearby habitats (Kasina *et al.* 2009). In open agricultural farms in Kakamega, insects such as social bees, thrips, ants and other biotic organisms are common visitors of green pepper flowers for their floral resources i.e. nectar and pollen. These feral pollinators of green pepper grown on farm lands in Kakamega provide an economic benefit to the farmers (Kasina *et al.* 2009). Almost 40% of the annual value owned by agriculture farmers in Kakamega represent the net returns derived from feral bees and other biotic pollinators (Kasina *et al.* 2009). This net return of the annual value obtained by the Kakamega farmers is attributed to the improvement of fruit quality through entomophilous pollination; thus enhancing their prices in the Kakamega market. Recently, there has been an increased demand on a global scale for efficient pollinators of green pepper grown in greenhouses (Cruz *et al.* 2005) which has become more evident with the advent of the colony collapse disorder (CCD) in honey bees (Steffan-Dewenter *et al.* 2005, Villanueva *et al.* 2005).

These disturbing issues thus necessitate the need for alternative pollinators to ensure global food security

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(Roubik 1995). Some stingless bee species have shown potential as candidate pollinators for commercial pollination in agricultural systems (Saraiva *et al*. 2003; Slaa *et al*. 2006). However, the pollination effectiveness of most stingless bee species is still unknown (Cruz *et al*. 2005). The pollination effectiveness of a specific stingless bee species depends very much on the crop species (Slaa *et al*. 2006). The use of stingless bee for crops pollination in the African continent is lacking due to knowledge gaps in the domestication of African stingless bee species (Raina *et al*. 2011). Over twenty species of stingless bees have been described in the African continent (Eardley 2004); among them *Hypotrigona gribodoi* Margrette, 1884 being the species reported to be frequently found nesting in wall crevices of human residential houses (Kajobe 2007). In western Kenya, *Hypotrigona gribodoi* is commonly found foraging on flowers of cultivated crops in homesteads, contrary to *Meliponula ferruginea* (reddish brown) Lepeletier, 1841 a species which also nests in wall crevices of human residential houses. This study was therefore conducted to investigate the pollination efficiency of *H. gribodoi* in improving the quality of *Capsicum annum* Linné, 1753 fruits and seeds.

MATERIALS AND METHODS

The experiments on pollination were carried out in plots of 4 m^2 replicated five times for each of the three treatments. All experimental plots were similar in size and located on a farmland neighboring the Kakamega forest at Isiekuti village in western Kenya. Treatment plots were completely covered with net cages (2 m × 2 m × 2.5 m) for self-pollinated plants and plants pollinated by *H. gribodoi*; while for unmanaged pollination (feral pollinators), plots were partially covered with a net cage to allow *Capscicum annum* flowers to be visited by other biotic organisms. Forty-five plants of green pepper variety California Wonder were transplanted into the five plots of each treatment. In all plots, plants were arranged in three rows, with a 0.65 m wide aisle between rows and 0.50 m spacing between plants. One hived colony of *Hypotrigona gribodoi* was introduced into five randomly chosen plots under net cages; while no bee colonies were introduced in the other five plots under net cages and served as controls (self-pollinated).

The number of daily visits made by forager bees of *H. gribodoi* on a same newly open flower under net cages was recorded by observing and recording bees visiting flowers during a standard period of time according to Dafni (1992). Data on flower visitation was collected from 6:30 am to 6:00 pm under sunny conditions, without rain. The number of visitation realized by *H. gribodoi* on a same newly opened green pepper flower was reported as the mean daily visit. The first two fruits produced by each plant in each plot were collected as soon as they attained maturity and their weights measured to the nearest gram. The seeds contained in each of these first fruits were counted and seed characteristics (length, width) recorded on twenty five randomly sampled seeds in each of the first two fruits produced per plant in each of the five plots for the three treatments. The total weight of seeds based on dry mass was recorded and the mean weight of a seed per treatment was determined. All data were presented as the mean ± standard error of the mean. The observed parameters were compared with one-way ANOVA; the Tukey test was used to separate significant means. The level for significance of difference was established as $P \leq 0.05$. All statistical analyses were done using R software version 2.14.0 (R Development Core Team 2005).

RESULTS

Hypotrigona gribodoi visited flowers of green pepper on plants in plots under net cages to collect pollen. The number of visits of *H. gribodoi* recorded daily on the same newly-opened flower averaged 8.6 ± 0.68. However, on a daily basis *H. gribodoi* visited the same newly-opened flower up to a maximum of twelve times and a minimum of five times.

Fruits and seeds were produced in all three treatments and the average weight of green pepper fruits obtained varied significantly among the three treatments ($F = 46.65$; dl = 2, 12; $P < 0.001$). Fruits were heavier in the green pepper plants whose flowers were pollinated by *H. gribodoi* and feral pollinators compared to fruits produced by self-pollinated flowers (Fig. 1). However, the fruits obtained from flowers pollinated by feral pollinators were lighter compared to those pollinated by *H. gribodoi*.

The average number of seeds in the fruits of green pepper also varied among the three treatments (Fig. 2). A significant difference was observed within the average number of seeds within fruits in the three treatments ($F = 18.91$; dl = 2, 12; $P < 0.001$). The average number of seeds was higher in the green pepper fruits whose flowers were pollinated by *H. gribodoi* and by feral pollinators compared to fruits whose flowers were self-pollinated. The fruits of green pepper obtained from flowers pollinated by feral pollinators had fewer seeds compared to those pollinated by *H. gribodoi*.

The average weight of a seed (dry mass) per fruit varied significantly among the three treatments ($F = 103.92$; dl = 2, 12; $P < 0.001$). The average weight

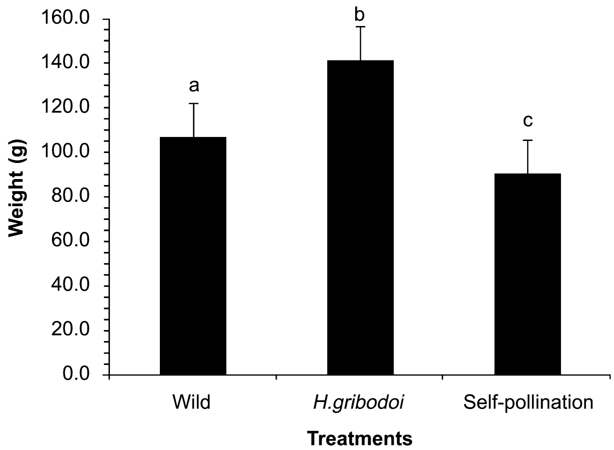

Figure 1 Average weight of green pepper fruits produced through pollination by feral pollinators, *Hypotrigona gribodoi* and self-pollinated flowers in a small scale farm vicinal to Kakamega forest, Western Kenya. Closed bars with different letters are statistically different at $\alpha = 0.01$.

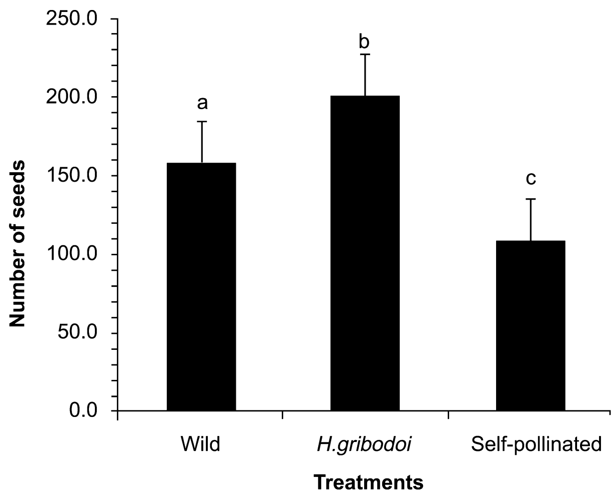

Figure 2 Average number of seeds produced in fruits of green pepper through pollination by feral pollinators, *Hypotrigona gribodoi* and self-pollinated flowers in a small scale farm vicinal to Kakamega forest, Western Kenya. Closed bars with different letters are statistically different at $\alpha = 0.01$.

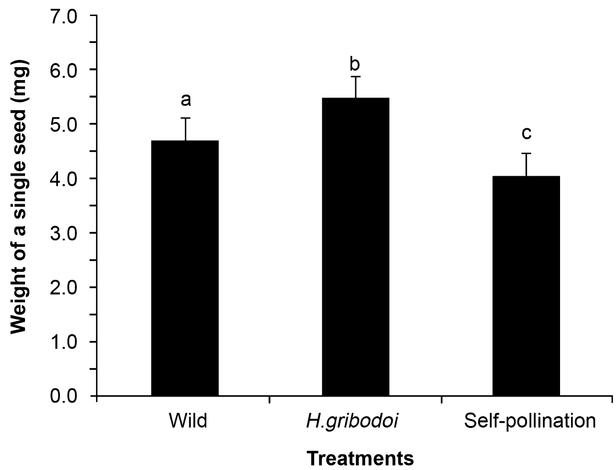

Figure 3 Average weight of seeds (dry mass) from green pepper fruits produced through pollination by feral pollinators, *Hypotrigona gribodoi* and self-pollinated flowers in net cage in a small scale farm vicinal to Kakamega forest, Western Kenya.

Table 1 Average length and width of seed contained in fruits of green pepper produced through pollination by feral pollinators, *Hypotrigona gribodoi* and self-pollinated flowers

Treatments	Number of plots	Seed length (mm)†	Seed width (mm)†
H. gribodoi	5	4.30 ± 0.029[a]	3.69 ± 0.042[a]
Feral pollinator	5	3.98 ± 0.006[b]	3.28 ± 0.012[b]
Self-pollinated	5	3.85 ± 0.006[c]	3.18 ± 0.007[b]

†Mean ± SE with values in each column followed by the same letter are not statistically different at $\alpha = 0.05$.

of a single seed based on its dry mass was heavier in the green pepper plant whose flowers were pollinated by *H. gribodoi* and by feral pollinators compared to green pepper plants seeds whose flowers were self-pollinated (Fig. 3). The average weight of a seed from fruits produced from flowers pollinated by feral bees was lighter compared to those pollinated by *H. gribodoi.*

The average length and width of a seed varied among the three treatments (Table 1). The average length of a seed produced was significantly different among all the three treatments ($F = 163.13$; $dl = 2, 12$; $P < 0.001$). Seeds were longer in fruits produced by green pepper plants whose flowers were pollinated by *H. gribodoi* and by feral pollinators. However, seeds from fruits of green pepper obtained from flowers pollinated by feral pollinators in the open field were shorter compared to seeds from fruits obtained from flowers pollinated by *H. gribodoi*. A significant difference was also observed in the average width of seeds from fruits produced among the three treatments ($F = 111.4$; $dl = 2, 12$; $P < 0.001$). The mean width of seeds in fruits produced from green pepper plants whose flowers were pollinated by *H. gribodoi* was higher compared to seeds in fruits of green pepper plants whose flowers were pollinated by feral pollinators or were self-pollinated. No differences were observed in mean widths among seeds from fruits of green pepper plants whose flowers were pollinated by feral pollinators or were self-pollinated. As such seeds contained in fruits produced by flowers pollinated by *H. gribodoi* were bigger than seeds in fruits produced by plant whose flowers were either pollinated by feral pollinators or were self-pollinated.

DISCUSSION

The aim of this study was to compare fruit and seed quality among green pepper plants grown in plots whose flowers are self-pollinated, pollinated by feral pollinators in the open field and those which were pollinated by *H. gribodoi* stingless bee species. It was observed that the heaviest green pepper fruits, highest number of seeds per fruit, heavier and bigger seeds were those which were produced from the flowers pollinated by *H. gribodoi* compared to the other two treatments. Differences in seeds per fruit due to floral visits by a specific pollinator are a direct consequence of the quantity of pollen grain deposited on stigmas of flowers visited by the pollinator (Serrano & Guerra-Sanz 2006). Moreover, it is reported that seeds play an important role in the fruit setting process, since poorly developed fruits are the result of an unequal seed distribution inside the fruit (Cruz *et al.* 2005). Thus, in a well-pollinated flower, a rapid development of ovary occurs, and the fecundated seeds produce plant growth hormones, leading to a good fruit development (Cruz *et al.* 2005). We suggest that deposits of pollen grains in stigmas might have been higher in green pepper flowers pollinated by *H. gribodoi*; thus resulting in setting of more seeds of better size and weight per fruit. As a consequence, fruit produced from flowers pollinated by *H. gribodoi* were bigger compared to the flowers either pollinated by feral pollinators or self-pollinated.

Pollination studies on green pepper using stingless bees have also been reported in other tropical regions of the world. For example, *Nannotrigona perilampoides* Cresson, 1878 in Mexico was found to be a good alternative pollinator to *Bombus impatiens* Cresson, 1863 in pollination of *Capsicum chinense* Jacquin, 1776 grown in greenhouses (Palma *et al.* 2008). The stingless bee *Trigona carbonaria* Smith, 1854 has been reported in Australia to be a pollinator of green pepper which increased fruit weight by 11% and the number of seeds per fruit by up to 34% when compared to self-pollinated crops (Occhiuzzi 2000). In Brazil, green pepper flowers pollinated by *Melipona subnitida* Ducke, 1911, produced fruits of better quality and the latter bee species could be use as an efficient pollinator to increase seed production as opposed to self-pollination (Cruz *et al.* 2005). Our findings corroborate previous studies that some species of stingless bees are promising alternative pollinator for managed pollination in agriculture. Furthermore, for the green pepper, although autogamous, floral visits by stingless bees were shown to contribute to improved fruit quality compared to self-pollination. *Hypotrigona gribodoi* is an efficient pollinator of green pepper which contributes to the improvement of green pepper fruit and seed qualities in western Kenya compared to feral pollinators and self-pollination. Thus, the utilization of *H. gribodoi* colonies in managed pollination of green pepper, such as in green houses, has the potential to increase the income of rural farmers as a result of enhancement of their prices on the local market due to improvement of green pepper fruit quality.

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