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ORIGINAL RESEARCH ARTICLE

African endemic stingless bees as an efficient alternative pollinator to honey bees in greenhouse cucumber (*Cucumis sativus* L)

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ABSTRACT
The current honey bee decline necessitates the use of alternative native pollinators to ensure global food security. Here, we compared the pollination behaviour and efficiency of the African honey bee (*Apis mellifera*) and six African endemic Meliponini (*Meliponula bocandei, Dactylurina schmidti, Meliponula lendliana, Hypotrigona gribodoi, Meliponula ferruginea* and *Meliponula togoensis*) in a greenhouse with the non-parthenocarpic cucumber variety *Super Marketer*. Honey bees and *D. schmidti* started foraging on introduction in the greenhouse, while *M. lendliana* and *M. togoensis* showed the longest delay. In most species, foragers collected nectar and pollen, excepting *M. bocandei* that specialized in nectar collection, and *H. gribodoi* and *M. togoensis,* specialized in pollen collection. African honey bees visited flowers the shortest, while *H. gribodoi* and *D. schmidti* had a 2-fold probing time, on average. Most stingless bees species had a lower hive activity with fewer foragers encountered per hour than for honey bees, except *D. schmidti*. *M. bocandei, M. ferruginea*, *A. mellifera scutellata* and *H. gribodoi,* yielded a seed germination percentage of around 90%. *M. lendliana*, *M. togoensis* and *D. schmidti* yielded a much lower seed germination percentage around 30%, which indicates that the quality of pollination was remarkably lower by using these three species. The highest sugar content was recorded in fruits from flowers pollinated by *M. bocandei,* African honey bees*, D. schimdti* or *M. togoensis* with the same solid content as the gold standard method, i.e., hand cross-pollination. We found that *M. bocandei* was the most efficient cucumber pollinator of all species tested: because pollination by this species yielded the largest and heaviest fruits and the highest seed numbers.

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Conservation; Meliponini; behaviour; pollination; cucurbits; crop yield

Introduction

Social bees such as the honey bee, *Apis mellifera* L., and bumble bees, *Bombus* spp., have been domesticated and are used as pollinators of many crops in the absence of suitable wild pollinators. Adequate pollination improves fruit and seed quality, thus increasing the economic empowerment of farmers through increased marketable income (Klein et al., 2007). In sub-Saharan Africa, *Apis mellifera* is the only commercially available pollinator for farmers because the use of bumblebees is mainly restricted to their geographical area of origin, thus excluding most of the African continent. Honey bees are currently facing dramatic declines in several regions of the world (Bauer & Wing, 2010; Potts et al., 2010; Roubik, 1995; Steffan-Dewenter et al., 2005; Villanueva et al., 2005), which triggers the search for other pollinators that are available locally to ensure food security (FAO, 2018). Furthermore, the use of native pollinators, other than honey bees, can be part of a conservation strategy to preserve the species diversity in the region where they occur. In Africa, other taxa of social bees, such as stingless bees, live in perennial colonies. Stingless bees are reported to be found only in tropical and subtropical regions of the world (Michener, 2000; Velthuis, 1997) and over 20 species have been reported so far on the African continent (Eardly, 2004).

The study of stingless bees as pollinators of food crops is more advanced in South and Central America. For example, the pollination efficiency of stingless bee species was demonstrated in crops, such as habanero pepper (Cauich et al., 2006), bell pepper (Oliveira et al., 2005), squash (Meléndez et al., 2000), and tomato (Santos Dos et al., 2009). Despite the increasing effort in America and Asia to study stingless bees as pollinators under greenhouse conditions, their pollination effectiveness remains

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largely unknown for many crops (Azmi et al., 2019; Cruz et al., 2005; Meléndez et al., 2018). The pollination behaviour and efficiency of African stingless bees under greenhouse conditions remain largely unstudied. Stingless bee species are not yet used for crop pollination on the African continent due to knowledge gaps in the domestication of African stingless bee species (Raina et al., 2011). Therefore, this study aimed at gaining insights into the foraging behaviour and pollination efficiency (fruit and seed quality of the crop) of six stingless bee species (*Dactylurina schimdti* (Stadelmann, 1895), *Meliponula bocandei* (Spinola, 1853), *Meliponula togoensis* (Stadelman 1895), *Meliponula lendliana* (Friese 1900), *Meliponula ferruginea* (Lepeletier, 1841) and *Hypotrigona gribodoi* (Magretti, 1884)) by comparing their behaviour and efficiency in setting fruit quality to the African honey bee (*Apis mellifera scutellata* Lepeletier, 1836**).**

In greenhouse non-parthenocarpy cucumber farming, *A. mellifera* has so far mostly been used as a pollinator because it yields better quality fruits than self-pollinating cucumber plants (Couto & Calmona, 1993; Santos Dos et al., 2008). The fruit quality and seed production in non-parthenocarpic cucurbit plants depend strongly on the pollinator species used (Collinson, 1976; Meléndez et al., 2002; Stanghellini et al., 1997). Pollination leads to flower fertilization and subsequent fruit and seed development. The efficiency of pollination will determine the number of deposited pollen grains on the stigma and influences the number and distribution of seeds within the fruit, which then influence fruit quality (Dražeta, 2002; Ward et al., 2001) and quantity (Garratt et al., 2013, 2014; Stephenson, 1981).

Cucumber, *Cucumis sativus* L, is a species in the gourd family, Cucurbitaceae (Nonnecke, 1989; Robinson & Decker-Walters, 1997). It is a widely cultivated creeping vine that is grown for its cylindrical fruits, which are a good source of vitamin C (Nitsch et al., 1952; Schaffer & Paris, 2003; Tanurdzic & Banks, 2004). Cucumbers have been cultivated for several thousand years and have spread from the foothills of the Himalayas of Nepal to neighbouring eastern and southern Asia, and later to Europe and Africa (Schaffer & Paris, 2003). In Africa, cucumber cultivation in greenhouses is becoming more and more widespread. A standard cucumber cultivar plant is monoecious, which means that it bears both female and male flowers. Such a cultivar will typically require a pollen vector to spread their pollen from their male flowers to their female flowers to set fruit. In almost 90% of angiosperms, pollination is facilitated through insect vectors, mainly bees (Kevan & Baker, 1983; Michener, 2007; Ollerton et al., 2011). As male and female cucumber flowers produce ample volumes of sweet nectar (Collison, 1973), bees are their main pollinators (Barber et al., 2011). However, the use of greenhouse closures or adverse weather conditions would prevent flower visitation by bees. Parthenocarpic cucumber cultivars have also been developed to dismiss the need for pollinators in closed environments, thus obtaining seedless fruit without pollination (Mensah & Kudom, 2011; Richards, 2001). However, parthenocarpy cultivars produce seedless fruit in the absence of bee pollination and if pollinated the plants will yield normal seeded fruits but the fruit may be off-shaped (Valenzuela et al., 1994). The use of different cultivars is highly dependent on the region. In Africa, cucumber production is impeded by the limited use of improved cultivars (Afari-Sefa et al., 2012). Besides, local consumers' preferences push growers to use greenhouse and non-parthenocarpic varieties, which implies the need to assure pollination in cucumber farming for adequate fruit yield and quality production. Stanghellini et al. (2002) reported that for the same plant, some bee species such as bumble bees have been shown to be more efficient pollinators than others. Therefore, we aimed at identifying the most efficient native stingless bee pollinator of a non-parthenocarpic cucumber cultivar in a closed greenhouse. These results will contribute to the knowledge and use of African endemic stingless bees as pollinators to improve fruit production of greenhouse cucumber.

Materials and methods

Study site

The study was conducted in greenhouses at the African Reference Laboratory for Bee Health of the International Centre of Insect Physiology and Ecology in Nairobi, Kenya (01 13 25.3 S, 36 53 49.2 E and altitude 1,600 m.a.s.l). The experimental greenhouses measured 8 m in width and 24 m in length (area= 192 m^2), and had a semi-spherical top section of a maximum height of 3.5 m. The top cover material of the greenhouse was made of polythene plastics translucent film and its sides were closed with polythene mesh which both reflects the available sunlight and reduces shadows. Each greenhouse was divided into 8 plots of 3 m width and 8 m length (area= 24 m^2). The inner tunnel was partitioned longitudinally using a polythene mesh 50 Anti-insect net; which is smaller than the body width of *H. gribodoi*, the smallest stingless bee species used in this study. During the experiment, temperature ranged from 19 to 39.5 C, humidity from 38 to 79% and light intensity from 1.23 to 34.89 klux, between 6:00 am and 18:00 pm.

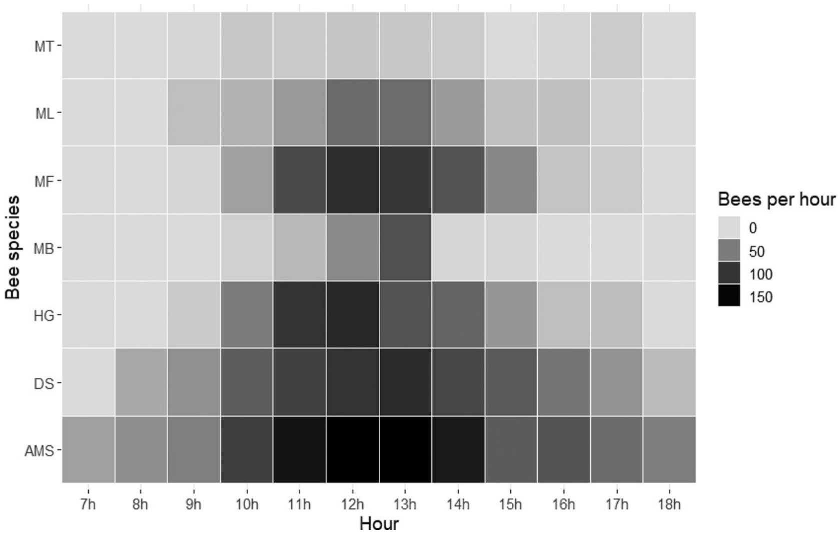

Figure 1. Foraging pattern of foraging workers of stingless bee species and *A. mellifera scutellata* and peak foraging activity as measured using a digital counter of bees foraging on flowers in a one-minute count. All points represent the mean_SE of bees/min/h the plots from two replicates per species over 10 sample days in greenhouses.

Study species

Plant

Cucumber (*Cucumis sativus* L) Super Marketer variety was bought from Amiran Kenya Ltd. and was used in greenhouses. This is a pollinator-dependant cucumber variety that matures relatively fast in about 1–2 months. The variety produces high fruit yields (16–32t/ha) and fruits are dark green in colour, with thin skin and firm flesh. It grows well in hot climatic conditions and is found to be very popular in the market (East African Seed Co. Ltd., 2019). Male and female cucumber flowers are crinkly and yellow. The male cucumber flower grows on slender stems in clusters; each flower has three slender stamens with pollen at the end. Each female flower grows alone and has one ovary in the shape of a tiny cucumber at its base.

Bees

We evaluated the pollination of cucumber provided by seven bee species: *M. bocandei*, *M. togoensis*, *M. ferruginea*, *D. schmidti*, *M. lendliana*, *H. gribodoi* and *A. mellifera scutellata* (Supplementary figure 1). Colonies of the stingless bee species were taken from the demonstration meliponary located in the ICIPE (International Centre of Insect Physiology and Ecology, Nairobi, Kenya) headquarters (1.22376˚S and 36.89732˚E). They were all domesticated in hives developed by the *icipe*, except for *D. schmidti* whose colony was a wild nest (Supplementary figure 2). For *H. gribodoi,* the *icipe-1H* wooden hives (45 cm (L) x 15 cm (l)) was used, and for the *Meliponula* spp. that nest in tree cavities *icipe-5M* wooden hives (45 cm (L) x 18 cm (l) x 22.5 cm (H)) were used. For underground nesting stingless bee species, *icipe-1 clay pot hive* (20 cm (L) x 20 cm (l) x 45 cm (H)) were used. Nests of the bee species were harvested from the wild by rural farmers living in the surrounding of the Kakamega forest (Western Kenya region, 1.71800˚N and 34.511319˚E) and were transferred in the *icipe* hives. *D. schmidti* nests were harvested in farmlands in Kilifi, Kenya costal region (3.6305°S and 39.8499°E**).** Except *D. schmidti,* these stingless bee species are being domesticated by farmers for their honey, which they use for income generation, food, traditional medicine, and rituals. For the honey bees we used colonies that colonized a 5 frames small size designed Langstroth hive (Hive body: 25 cm x 22.5 cm) x 22.5 cm; Shallow: 25 cm x 22.5 cm) x 15.5 cm) made for pollination experiments in greenhouses.

Like *A. mellifera scutellata*, the stingless bee species are all social bee species living in peri-annual colonies. The number of individuals in a well-established colony varies within the species and strongly decreases proportionally with the bee species body size (bee species with larger body sizes are typically more populous). According to Wondmeneh et al. (2020), the population size of some stingless bee colonies such as *M. beccarii* (body size 5.8 mm) with an average nest volume of 8,935.3 cm^3 relates to approximate 6,000 individuals. *M. bocandei* is a large bee species (7 mm long) and constructs nests in cavities in trees and brood combs are organized in a cluster (Eardly, 2004; Michener, 2000). *M. togoensis* and *M. ferruginea* have a medium body size (5.9 mm long) and construct nests in cavities in trees with horizontally arranged brood combs (Eardly, 2004; Michener, 2000). *D. schmidti* has a small body size (5 mm long) and constructs exposed nests that hang on tree branches with brood combs arranged vertically (Eardly, 2004; Michener, 2000). *M. lendliana* is a small bee (4 mm long) that builds its nest in underground voids with horizontally

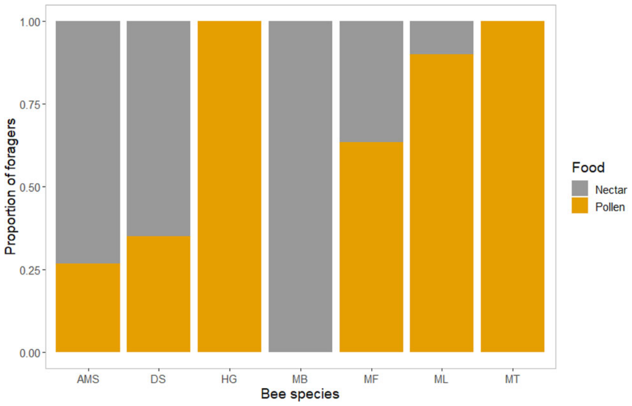

Figure 2. Proportion of forager bees for each six stingless bee species and honey bees collecting nectar or pollen on cucumber flowers.

organized brood combs (Eardly, 2004; Michener, 2000). *H. gribodoi* is a very small bee species (2 mm long) and constructs nests in cavities in trees or walls and brood combs are organized in a cluster (Eardly, 2004; Michener, 2000). In this experiment, the selection of colonies within species was conducted in such a manner that they had an approximate similar population size. Therefore, hives of colonies of *M. togoensis*, *M. ferruginea* and *M. lendliana* were selected based on the same number of brood layers in the nest (10 layers of brood combs) and same nest volume. For *D. schmidti*, colonies used were of the same nest volume as those of *M. togoensis*, *M. ferruginea* and *M. lendliana*. *M. bocandei* colonies in hives were selected for those whose brood and nest volume were approximatively like those of the other previous stingless bee species. To use *H. gribodoi* colonies which may contain around 3,000 to 4,000 individual bees we selected for 4 years old colonies that were very populated and easily recognized by those whose nest volume was approximatively similar to the brood volume of the other big body size stingless bee species. Colonies of *Apis mellifera* used were kept in a small size Langstroth hive designed to contain less than 5,000 individual bees. Food stored in hives by the selected colonies was harvested a week earlier to their introduction in the greenhouse to minimize the influence of the amount of stored food on the foraging behaviour of the individual bees and the colony.

When 5% of plants started to bloom in a specific plot, one colony of a bee species was introduced into one plot in each greenhouse.

Experimentation

Experimental setup

Three types of pollination were tested, namely no pollination, artificial pollination: hand self-pollination and hand cross-pollination, and bee pollination. The bee pollination was tested using the African honey bee (*A. mellifera scutellata*) and 6 stingless bee species (*D. schimdti, M. bocandei, M. togoensis, M. lendliana, M. ferruginea* and *H. gribodoi*).

Each greenhouse was divided into 8 plots of 3 m width and 8 m length (area= 24 m^2) partitioned by polythene meshes (Supplementary figure 3). Per greenhouse, seven plots were used for accommodating one of the bee species and the 8th plot was used to accommodate self-pollination and cross-pollination treatments. A total of 240 seedlings of cucumber were transplanted in black plastic planting bags (23 litres capacity) per greenhouse. Each plot received 30 seedlings of cucumber arranged in 3 rows of 10 plants per row in a triangular planting pattern with 0.80 m spacing between plants in the same row and 0.90 m spacing between plants of different rows in each plot (Supplementary figure 4). All treatments were replicated twice, once in greenhouse A and once in greenhouse B. The crops were watered twice a day, early in the morning and late in the evening.

To assess the impact of "no pollination" (control), artificial pollination (hand self-pollination and hand cross-pollination) as treatments in a single plot per greenhouse, we bagged a total of 90 floral buds ($N = 1$ flower bud/plant * 30 plants * 3 treatments * 1 plots *1 greenhouse). Bagged female flowers ($N = 30$ flowers = 1 flower/plant * 30 plants) that were not exposed to any pollination were classified as control (NP, no pollination). To obtain artificial self-pollinated flowers from previously bagged floral buds, mature flowers ($N = 30$ flowers= 1 flower/plant * 30 plants) were hand-pollinated with pollen from a male flower taken from the same plant, then bagged again and tagged as hand self-pollinated (HSP). We proceeded similarly to obtain artificial cross-pollinated flowers ($N = 30$ flowers= 1 flower/plant * 30 plants), but this time pollen was taken from a male flower of a different plant, then bagged again and classified as hand cross-pollinated (HCP).

Bee behaviour

The time until the onset of foraging behaviour for each bee species on cucumber flowers was here

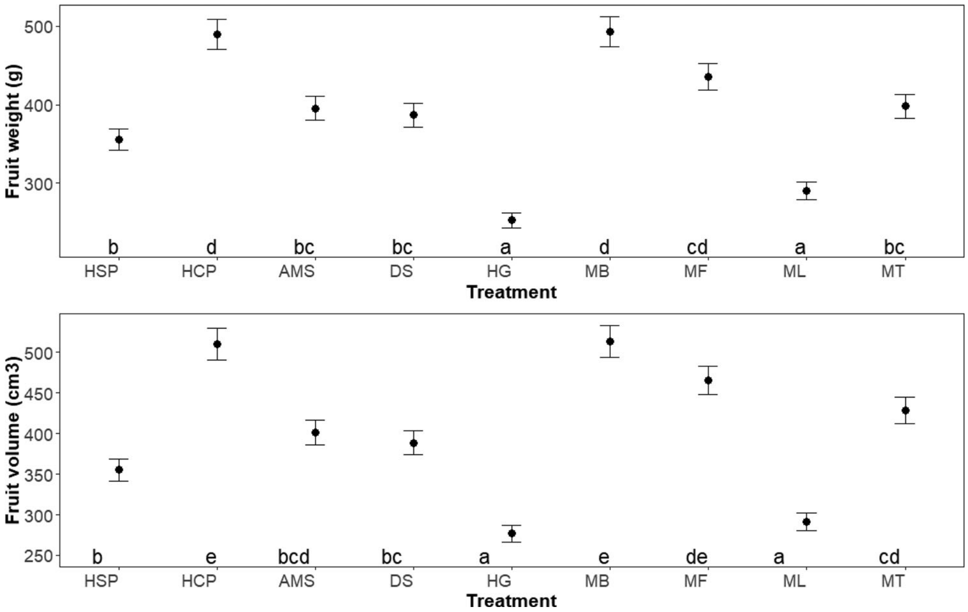

Figure 3. Average fruit weight and fruit volume (model adjusted mean ± SE) per treatment category. Different letters in graph depict means that were significantly different at $P < 0.05$.

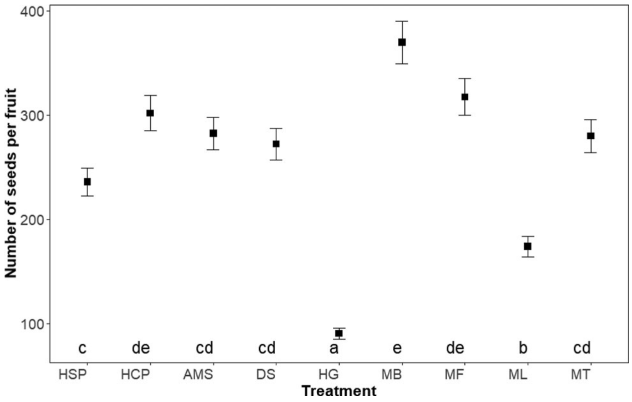

Figure 4. Number of seeds per fruit (model adjusted mean ± SE) that were obtained by different pollination treatments. Different letters depict means that are significantly different at $P < 0.05$.

expressed as the number of days from the moment colonies were introduced in their respective blooming plot in the greenhouses to the moment forager bees start foraging. This was conducted by observation on daily basis until the day a single forager was identified collecting nectar or pollen on flowers in their respective plots in the two greenhouses, respectively.

The flight activity in stingless bees includes waste removal from the colony and the collection of resin, latex, leaves, water, fungi spores, trichomes, fragrances, oils, seeds, animal feces, clay, besides nectar or pollen collection (Eltz et al., 2002; Hilario et al., 2001). Therefore, to assess the bee species foraging activity and peak foraging time for pollen and nectar across the day we counted the total number of bees per colony foraging on flowers per treatment and per greenhouse within a 1-minute count in an interval of 60 minutes (hourly) between 08:00 to 18:00. These observations were made on sunny days or 60% cloudy over ten consecutive sampling days. The number of bees was registered using a digital counter (Klein et al., 2007).

The food resource collected by each bee species was determined by counting the number of foragers collecting nectar and pollen by observing which food resource was collected on flowers. Nectar gathering foragers were recognized by individual bees dipping their proboscis into the base of the flower corolla while pollen foragers were identified as individuals scrabbling over the anthers (Keith et al., 2013).

The foraging position of the bees during nectar and pollen collection was simultaneously documented when recording which food resource is being collected by each bee species. The foraging position was recognized by observing whether the bee approached the anther or nectaries either by climbing (top working bees), either through the anthers and stigma respectively or either from the side (side working bees) of the flowers (Keith et al., 2013).

Table 1. The nine levels of the factor "Treatment" used in this study excepting for fruit weight and volume, where data were available for "no pollination" level.

Treatment level	Abbreviation
No pollination	NP
Hand self-pollination	HSP
Hand cross pollination	HCP
Apis mellifera scutellata	AMS
Dactylurina schimdti	DS
Hypotrigona gribodoi	HG
Meliponula bocandei	MB
Meliponula ferruginea	MF
Meliponula lendliana	ML
Meliponula togoensis	MT

The probing time on flowers during foraging was also recorded simultaneously when collecting information on the food resource and foraging position of each bee species. Probing time was determined by recording the time a bee spends from the moment of dipping its proboscis in the flower corolla until the moment of its leave (Kiatoko et al., 2014; Santos Dos et al., 2008). For each of the three last bee behavioural parameters, observation was conducted on a single day on same 30 flowers (1 flower/plant * 30 plants) randomly selected per plot and per greenhouse. A total of 210 flowers (30 flowers/plot * 7 plots * 1 greenhouse) was observed per greenhouse.

Pollination efficiency: fruit parameters

To determine the fruit quality obtained from each type of pollination; we used the same 30 fruits to measure the average fruit weight, average fruit length, average fruit volume, and average fruit total soluble solids content (Nkansah et al., 2012). Additionally, the same 30 fruits were used to compare the total amount of average mature seed set, average seed weight, average seed width, average seed length, average seed thickness, average seed volume and average seed germination percentage. Fruit weight was determined by weighing fruits to the nearest 0.01 g using an electronic balance scale (Kiatoko et al., 2014). A flexible measuring tape was used to measure fruit length (Keith et al., 2013; Kiatoko et al., 2014; Nkansah et al., 2012; Santos Dos et al., 2008). Fruit volume was determined through the water displacement method, whereby each fruit was placed in a graduated cylinder (2000 mL) containing a known volume of water (initial volume). Based on the difference between the final volume (after immersion of the fruit) and the initial volume, fruit volume was obtained (Manfio et al., 2011). Sweetness of cucumbers can be easily, quickly, and objectively assessed by using a refractometer to determine soluble solids content of juice squeezed from the fruit flesh (Thompson & Kelly, 1957). Therefore, the effect of pollination type on the fruit total soluble solids content was determined using a digital pocket food sugar refractometer model ATAGO POCKET PAL-1 (Keith et al., 2013; Nkansah et al., 2012).

Pollination efficiency: seed parameters

The average amount of mature seed set was determined by counting the number of seeds present in fruits obtained with each type of pollination (Kiatoko et al., 2014; Primack, 1987). The average seed weight to the nearest milligram (Primack, 1987) was measured by randomly selecting 10 seeds in fruits from each type of pollination using an electronic balance scale (Kiatoko et al., 2014). Seed length, width, thickness, and volume were also measured from the 10 randomly selected seeds from fruits from each type of pollination. Cucumber seeds have a prolate ellipsoid shape and therefore their volume was calculated by the formula $V = \frac{4}{3}\pi\left(\frac{x}{2}\right)\left(\frac{y}{2}\right)\left(\frac{z}{2}\right)$; with V equal to seed volume, π equal to 3,14, x equal to seed length, y equal to seed width and z equal to seed thickness.

Seeds were nursed in seed nursery raising trays and planting media as a substrate to nurse the seeds (Abdel-Haleem & El-Shaieny, 2015; Nkansah et al., 2012). For each type of pollination, 300 mature seeds were randomly selected, and germinated seeds were counted after 10 days (Kader, 2005) and each treatment was replicated twice. A total of 10 days was used to count the number of seeds germinating daily to estimate the mean germination time of seeds per pollination treatment (Kader, 2005).

Data analysis

All analyses were carried out in R 4.0.0 (R Development Core Team, 2015). The number of days before different bee species started foraging was analysed by a Kruskal–Wallis test, followed by a Dunn test to check pairwise differences among bee species.

We calculated contingency tables to investigate whether the number of visits assigned to nectar or pollen differed between treatments. Significance was estimated using a Chi-squared test.

For the rest of the variables, Generalized Linear Models (GLMs) were performed by declaring Treatment and Greenhouse, and their interaction, as fixed factors. Just for seed weight there was an effect of the greenhouse and interaction term. Therefore, for all remaining variables, the effect of the greenhouse was declared in a new model as random effect by conducting mixed models in which treatment was the only fixed factor. The factor "Treatment" had 9 levels (see Table 1). A post hoc Tukey test was conducted for each model to see pairwise comparisons. Results were displayed back transformed to the original scale of the data.

Table 2. Average probing time (model adjusted mean ± SE) in cucumber flowers for each bee species, sorted from shortest to longest.

Bee species	Probing time (seconds)
A. mellifera scutellata	6.68 ± 0.334 a
M. bocandei	8.37 ± 0.373 b
M. ferruginea	8.70 ± 0.381 b
M. togoensis	8.87 ± 0.384 b
M. lendliana	10.92 ± 0.427 c
H. gribodoi	13.05 ± 0.466 d
D. schmidti	13.07 ± 0.467 d

Different letters indicate means that were significantly different at $P < 0.05$.

The variables "Seed number per fruit" fitted a negative binomial distribution. Seed weight was transformed by subtracting the mean from the absolute value of each observation, and then analysed as a normal distribution. Fruit weight, total solid content and volume fitted a gamma distribution. Fruit length fitted a normal distribution. Probing time (in seconds) per bee visit followed a Poisson distribution.

The foraging pattern as the number of bees foraging on flowers per one-minute count/hour across the day was compared among bee species by using a Poisson regression in which bee species was listed as fixed effect and hour of the day as random factor.

Germination variables were computed using R package "GerminaR" (Lozano-Isla et al., 2019), and two-way ANOVAs were used to test the effect of Treatment and Greenhouse on GRP (Germination percentage) and MGT (Mean Germination Time).

Results

Bee behaviour

Time until the onset of foraging behaviour

Once installed in the greenhouse, different bee species showed a slightly different delay to foraging on the cucumber plants (χ^2=12.48, df = 6, P = 0.052). *A. mellifera scutellata* and *D. schmidti* showed similar foraging responses ($Z = -0.36$, $P = 0.719$), with a delay of just 1.5 ± 0.7 and 2.5 ± 0.7 days, respectively (median ± SD). On the contrary, *M. lendliana* (13.5 ± 0.7 days) and *M. togoensis* (12.0 ± 1.4 days) were both characterized by a delayed foraging response, and correspondingly the number of days to start foraging significantly varied between these two species (*M. lendliana*, *M. togoensis*) and the two fastest ones (*A. mellifera scutellata* and *D. schmidti*, see Table S1 for paired differences).

Bee species foraging activity and peak foraging time on flowers

The bee species showed differences in their hourly foraging activity in the greenhouse here indicated by the number of bees counted in a 1-minute interval per hour (χ^2 = 1177.3, $P < 0.001$). The bee species *M. bocandei* (8.44 ± 2.36), *M. togoensis* (3.15 ± 0.94) and *M. lendliana* (14.50 ± 3.98) had an average rate of less than 15 bees per minute/hour and we found higher bee counts at noon for *M. lendiana* (Figure 1). *M. ferruginea* and *H. gribodoi* were characterized by intermediate levels of foraging frequency (around 27 bees per minute/hour) and they were mostly found foraging between 10:00 am to 15:00 pm (Figure 1). Other species were found foraging at a narrower time interval, such as *H. gribodoi* or *M. ferruginea* (Figure 1). On the contrary, *D. schimdti* and *A. mellifera scutellata* were characterized by high frequency of bees foraging on flowers that was maintained all day long (Figure 1).

Food resource

Bee species visiting cucumber flowers differed in their foraging preferences towards nectar or pollen (χ^2= 234.74, df = 6, $P < 0.001$). *A. mellifera scutellata*, and *D. schmidti* foragers mostly retrieved nectar, while *M. ferruginea* and *M. lendliana* collected mostly pollen. Unlike the four other species, *M. bocandei* only collected nectar, while *H. gribodoi* and *M. togoensis* only foraged on pollen (Figure 2).

Foraging position and probing time on flower

Honey bees and stingless bees were all observed approaching flowers from the top. Probing time in cucumber flowers differed among bee species (χ^2 = 213.67, $P < 0.001$). Flower visits by *A. mellifera scutellata* were the shortest, with an average probing time of 6.7 ± 0.3 seconds per flower (Table 2). Most *Meliponula* species (*M. bocandei*, *M. ferruginea*, *M. togoensis*) visited flowers during a similar timespan, around 8 seconds, except for *M. lendliana*, whose visits were longer (Table 2). *H. gribodoi* and *D. schmidti* probed cucumber flowers during for more than 13 seconds, on average (Table 2).

Pollination efficiency: fruit and seed parameters

Fruit weight and volume

The average fruit weight varied between treatments (χ^2 = 268.71, $P < 0.001$), ranging from nearly 500 g in the reference treatment (hand crossed pollination) and *M. bocandei* to less than 300 grams in *H. gribodoi* and *M. lendliana*-pollinated plants (Figure 3a). Same results were obtained for each treatment level for fruit length (results not shown). Fruit volume also varied among treatments (χ^2 = 280.38, $P < 0.001$) and yielded similar trends (Fig 3b). All results together, pollination by *M. bocandei* resulted in cucumber fruits of highest quality. *A. mellifera scutellata* fruit weight and volume were smaller to that of *M. bocandei* but similar to that of *D. schmidti, M. ferruginea* and *M. togoensis.*

Table 3. Pairwise comparisons (Z values) for total solid content among treatment levels.

Treatment (°Brix, mean ± SE)	HSP	HCP	AMS	DS	HG	MB	MF	ML	MT
HSP (4.04 ± 0.0511)									
HCP (4.23 ± 0.0535)	2.61								
AMS (4.33 ± 0.0549)	**3.98**	1.37							
DS (4.49 ± 0.0568)	**5.88**	**3.27**	−1.90						
HG (3.78 ± 0.0478)	**3.67**	**6.28**	**7.65**	**9.56**					
MB (4.27 ± 0.0540)	−3.14	−0.53	0.84	2.75	−**6.81**				
MF (4.06 ± 0.0514)	−3.07	2.25	**3.62**	**5.52**	−**4.04**	2.77			
ML (4.01 ± 0.0507)	0.42	3.03	**4.40**	**6.30**	−**3.25**	**3.56**	0.79		
MT (4.22 ± 0.0534)	−2.46	−0.15	1.52	**3.43**	−**6.13**	0.68	−2.09	−2.88	

Bold numbers indicate significant differences at $P < 0.05$. First column indicates the average total solid content and SE for each treatment.

Table 4. Average seed weight for each treatment (listed alphabetically) level and greenhouse (model adjusted mean ± SE).

Treatment	Greenhouse A	Greenhouse B
A. mellifera scutellata	8.34 ± 0.117 g	7.64 ± 0.117 g
D. schmidti	1.42 ± 0.117 d	4.06 ± 0.117 e
HCP	5.22 ± 0.117 f	6.15 ± 0.117 f
HSP	5.49 ± 0.117 f	5.14 ± 0.117 f
H. gribodoi	3.75 ± 0.117 e	5.70 ± 0.117 ab
M. bocandei	4.37 ± 0.117 c	3.02 ± 0.117 d
M. ferruginea	4.96 ± 0.117 b	6.04 ± 0.117 a
M. lendliana	5.22 ± 0.117 b	5.51 ± 0.117 b
M. togoensis	6.16 ± 0.117 a	4.34 ± 0.117 c

Different letters within each greenhouse group denote significant differences at $P < 0.05$.

Fruit soluble solids content

Pollination treatment influenced cucumber total soluble solid content ($\chi^2 = 128.22$, $P < 0.001$). The use of *A. mellifera scutellate*, *D. schimdti*, *M. togoensis* and *M. bocandei* yielded fruits with the same solid content as the gold standard method, hand cross-pollination (Table 3). All results together, pollination by *H. gribodoi* resulted in cucumber fruits with the lowest soluble solids contents.

Number of mature seeds

The number of seeds produced per cucumber fruit fluctuated among pollination treatments ($\chi^2 = 449.51$, $P < 0.001$). The use of *M. bocandei* again exceeded the number of seeds that were produced by the gold standard, hand cross-pollination, and all the other treatments (Figure 4). Consistent with previous findings on fruit quality, the lowest seed counts were recorded in fruits from flowers pollinated by *H. gribodoi* or *M. lendliana* (Figure 3).

Seed weight and volume

Seed weight varied among treatments ($F_{8,5391} = 305.51$, $P < 0.001$), greenhouses ($F_{1,5398} = 29.21$, $P < 0.001$) and the interaction among both factors ($F_{8,5382} = 81.79$, $P < 0.001$). Despite of greenhouse-caused differences, *A. mellifera scutellata* consistently yielded heavier seeds, and cucumbers obtained by *D. schmidti* pollination were characterized by light seeds (Table 4).

Pollination treatment significantly affected other seed quality parameters such as seed volume ($F_{8,5390}$=1127.3, $P < 0.001$). Interestingly, seed volume was higher in our two reference treatments, with no differences between hand self (3.413 ± 0.006 mm^3, mean ± SE) and hand crossed pollination (3.409 ± 0.006) for this variable ($Z = -0.442$, $P = 1.000$). The lowest values of seed volume were obtained, on average, for two *Meliponula* species, namely *M. lendliana* (2.895 ± 0.006) and *M. togoensis* (2.877 ± 0.006) ($Z = 2.213$, $P = 0.967$). Rest of species were characterized for intermediate values of seed volume among these two extremes (results not shown).

Seeds germination rate

The germination percentage varied among pollination treatments ($F_{8,10} = 60.02$, $P < 0.001$) but no significant differences were observed between greenhouses ($F_{1,17} = 4.14$, $P = 0.076$). Germination percentages could be clearly separated between two groups. while *D. schmidti*, *M. lendliana* and *M. togoensis* treatments were characterized by germination percentages around 30%, the rest of the treatments all yielded germination percentages around 90% (Table 5).

The use of different pollination methods leads to significant differences in MGT (Mean Germination Time) ($F_{8,10} = 453.75$, $P < 0.001$). In this case, there were also differences between greenhouses (($F_{1,17} = 7.80$, $P = 0.023$), being MGT values for each treatment slightly longer at greenhouse B. Paired contrasts were significant at all instances excepting *M. lendliana* and *M. togoensis*, which have the highest MGT, and HSP and *A. mellifera scutellata*, which were characterized by the lowest MGT, on average. Moreover, such differences might be inferred from the daily evaluation of germination percentages (Figure 5), which lead again to the differentiation of two main groups, as above: *D. schmidti*, *M. lendliana* and *M. togoensis* could be separated from the rest by their delayed germination response.

Discussion

Pollination is a critical ecosystem service for global food production, food security and economy (FAO, 2018). Honey bees are considered essential crop pollinators that meet agricultural needs and hive products (Chen & Siede, 2007). Over the past decades, honey bee declines have pointed to the need for alternative pollinators to assure global food security (Bauer & Wing, 2010; Potts et al., 2010; Roubik, 1995; Steffan-Dewenter et al., 2005; Villanueva et al., 2005). Furthermore, the use of native pollinators, other than honey bees, can be part of a conservation strategy. In Africa, knowledge on the use of African endemic stingless bee species is largely lacking due to a knowledge gap in their domestication. There are more than 20 African endemic stingless bee species, yet very little is known about their foraging behaviour and pollination efficiency compared to honey bees. Therefore, we compared the foraging behaviour of six native stingless bees to honey bees. We also assessed pollination efficiency of each bee species by comparing their effect on cucumber fruit and seed quality parameters between each other and to compare this to artificial pollination treatments (hand cross-pollinated and hand self-pollinated).

Bee behaviour

We observed multiple behavioural differences between the tested species. A. *mellifera scutellata and* D. *schmidti* started foraging the soonest after introduction in the greenhouse, specifically compared to species of the *Meliponula* and *Hypotrigona* genus. A similar tendency had been observed on sweet melon flowers in greenhouses where *A. mellifera scutellata* and *D. schmidti* started foraging sooner (4 days) once introduced in the greenhouse, while the *Meliponula* species, such as *M. lendliana* and *M. togoensis*, started to forage on melon flowers after approximately two weeks later (Kiatoko et al., submitted). Pollination studies on cucumber using Neotropical stingless bees in South America also found differences in time before the onset of foraging behaviour between honey bees and stingless bees on cucumber flowers in greenhouses (Nicodemo et al., 2013). Additionally, *Melipona quadrifasciata* showed promising results in increasing the production of tomato fruits and seed quality in a greenhouse environment in Brazil (Silva-Neto et al., 2019). We suggest that the difference observed in time before the onset of foraging behaviour between stingless bees and honey bees may be explained by differences in their ecology and learning ability. According to Henske et al. (2015), unlike the stingless bees that are exclusively adapted to tropical and sub-tropical regions, honey bees have furthermore adapted to various habitat types and climatic conditions such as temperate regions. This could indicate that honey bees are more flexible than stingless bees and can adapt quickly to very different ecosystems. Additionally, foraging success is largely dependent on olfactory cues or signals; and a study conducted on two African *Meliponula* stingless bees (*M.ferruginea, M. bocandei*) and *A. mellifera scutellata* indicated a difference in their learning ability, with a higher learning performance of honey bees than both stingless bee species (Henske et al., 2015). Similarly, Couto and Couto (2006) reported that honey bees are highly efficient at finding floral resources, which can explain the short time to foraging after introduction in the greenhouse.

Table 5. Mean (± SE) germination percentages per treatment (ordered from lower to higher).

Treatment	Mean ± SE
M. togoensis	29.33 + 0.33 b
D. schmidti	33.50 + 3.83 b
M. lendliana	33.67 + 6.00 b
M. ferruginea	87.17 + 2.17 a
HSP	89.83 + 2.50 a
A. mellifera scutellata	91.17 + 1.17 a
H. gribodoi	92.50 + 4.83 a
M. bocandei	93.33 + 0.33 a
HCP	95.67 + 9.67 a

Different letters denote significant differences among means at $P < 0.05$.

A. mellifera scutellata and *D. schmidti* had the highest number of bees foraging on flowers throughout the entire day but the peak of foraging activity in all bee species mainly occurred between 11:00 to 14:00, rather than early in the morning and

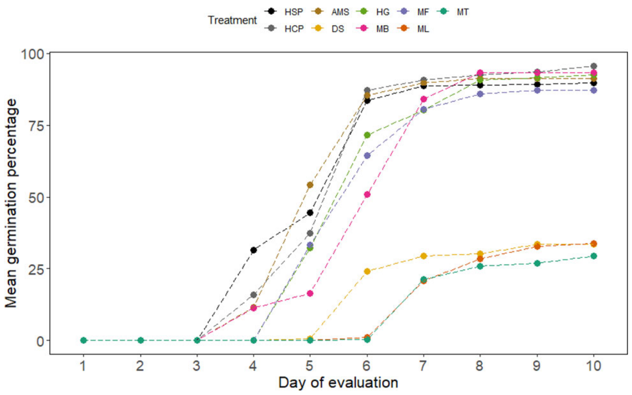

Figure 5. Average seed germination percentage (mean ± SE) per treatment along a 10-day long daily monitoring.

late afternoon. Temperature, light intensity, and relative humidity are reported among the main climatic factors that influence bee foraging activity (Nunes-Silva et al., 2010). They are also known to influence nectar resource availability (quantity and quality). We did not study the quantity or quality of the nectar throughout the day, but it is possible that foraging activity is highest when floral reward is too. Additionally, colony size within same and different bee species have been reported to influence foraging activity of bees (Danka et al., 1986; Eckert et al., 1994; Nunes-Silva et al., 2010). Here we used colonies of approximate same population size and very limited food stored in the hive in order to minimize the influence of colony population size and food store on the foraging activity of the bee species.

All seven bee species regularly visit both male and female cucumber flowers and all approached the flowers from the top during nectar or pollen collection. We suggest that this foraging position favours pollen to be deposited on the ventral side of the body, which is advantageous for pollination of cucumber plants because there is maximal contact with the ventral side of the bees' bodies and the stigma of female flowers. Furthermore, the bees cannot remove the pollen from the ventral midline by grooming behaviour because their legs cannot reach these so-called "safe sites" (Koch et al., 2017). When bees groom themselves and collect pollen in their corbiculae, these pollens can no longer contribute to flower fertilization (Thorp, 2000). Hence, pollen deposition on parts of the bees' bodies that cannot be removed by grooming and that ensure maximal contact with both the anthers and the stigma of the flowers will lead to an efficient pollination (Koch et al., 2017; Thorp, 2000). If pollen deposition occurs in other parts of the pollinator's body that do not correspond to parts that contact the stigma, pollen transfer for fertilization can be less efficient and results in a reduced fruit set, seed production and fruit quality (Armbruster et al., 2014).

However, bee species differed in their foraging preferences for nectar or pollen; with *M. bocandei* foragers observed only collecting nectar while *H. gribodoi* and *M. togoensis* were observed only collecting pollen compared to the four other bee species (Figure 2). The tendency of some stingless bee species to forage only on one specific food resource had been reported in some Neotropical stingless bees (Nicodemo et al., 2013; Santos Dos et al., 2008). For example, *Scaptotrigona* aff. *depilis* and *Nannotrigona testaceicornis* regularly visited flowers of three cucumber cultivars to collect only nectar and did not collect pollen. Many factors may explain bees' preference for nectar collection rather than pollen in cucumber. Some potential factors cause low pollen production and strong adherence of pollen to the stamen in some cucumber cultivars so that bees' interest in pollen collection is reduced (Nicodemo et al., 2013). Furthermore, it is also reported that there is a diverse specialization among stingless bee species for pollen or nectar (Roubik & Moreno Patiño, 2018). We suggest that *M. bocandei* specializes in nectar collection and *H. gribodoi* and *M. togoensis* specialize in pollen collection. We additionally found the shortest probing time during food foraging for *A. mellifera scutellata* compared to the stingless bees (Table 2). A similar observation was also reported between *A. mellifera* and *N. testaceicornis* a Brazilian stingless bee on cucumber flowers (Nicodemo et al., 2013; Santos Dos et al., 2008). Additionally, probing time was negatively correlated with the body size: larger bees (*A. mellifera scutellate*, *M. bocandei*, *M. ferruginea*, *M. togoensis*) had a shorter probing time than smaller bees (*H. gribodoi*, *M. lendliana*, *D. schmidti*) (Table 2). Harder (1983) reported that probing time is comprised of two-time components, namely access time and ingestion time. These are determined by the length of the bee's glossa, its body weight, the depth of the flower, the volume of the nectar and its viscosity. Probing time increases gradually with increasing depth for flowers shallower than the bee's glossa. It is plausible that honey bees have a short probing time due to their large body size (2.5 times larger than *M. bocandei,* the largest stingless bee in this study) and because of their longer glossae, shortening both the access time to reach the deep nectaries and the ingestion time. Shorter probing times were indeed also observed for the larger stingless bee species (*M. bocandei*, *M. ferruginea*, *M. togoensis*) compared to the small stingless bee species in this study. However, it is postulated that the shorter flower handling time entails a cost for foraging efficiency: there is limited movement on the flower, potentially leading to a lower distribution of pollen grains on the stigma lobes of flowers during a single visitation (Bomfim et al., 2014).

Pollination efficiency: fruit and seed parameters

Overall, the two stingless bees *M. bocandei* and *M. ferruginea,* the bee species with a larger body and colony size in this study, led to the highest cucumber fruit quality: these treatments had the largest and heaviest fruits with the most mature seeds. Interestingly, these two *Meliponula* species outperformed honey bees with regards to fruit weight, fruit volume and number of matured seeds and they yielded results comparable to the gold standard of hand cross-pollination (Figures 3 and 4). In addition, honey bees and *M. bocandei* also yielded a high total fruit solid content comparable to hand cross-pollination (Table 3). Seed germination rate, an indicator of vigour, was around 90% in all treatments; except for *D. schmidti*, *M. lendliana* and *M. togoensis* that were characterized by germination percentages around 30% (Table 5). These results therefore indicate that these three stingless bee species are not suitable pollinators of greenhouse cucumber. For plants that only propagate by seeds, seed production and a high seed germination rate are key for their establishment and for the crop yields (Barnard & Calitz, 2011; Delouche & Potts, 1983). Therefore, pollinators that yield low numbers of mature seeds or a low germination rate are considered unsuitable.

Together, these results demonstrate that *Meliponula* species can be efficient cucumber pollinators in greenhouses, even outperforming honey bees. *M. bocandei* in particular scores highest on many of the fruit quality parameters and yields results comparable to hand cross pollination.

Conclusions

Endemic African stingless bee species can be viable native alternative pollinators to honey bees for the pollination of greenhouse crops of considerable economic and social importance.

Here, we demonstrate that two *Meliponula* species, *M. bocandei* and *M. ferruginea*, which are endemic African stingless bee species found across western, central and east Africa are efficient pollinators of cucumber that outperform honey bees on fruit volume, weight and seed number. *M. bocandei*, in particular, shows to be the most promising cucumber pollinator. We also conclude that the use of endemic stingless bee species for pollination may contribute to the conservation of the species in the African continent.

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