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INSECT POLLINATORS

Performance, Effectiveness, and Efficiency of Honeybees as Pollinators of *Coffea arabica* (Gentianales, Rubiaceae)

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Abstract
Studies in crop plants analyzing floral biology in conjunction with effectiveness and efficiency of pollinators on pollen transfer and fruit formation are not common, although they are essential to provide better management actions. On this base, we selected a farm in Bahia, Brazil, to study pollination on coffee plants (*Coffea arabica* L.). Specifically, we want to analyze if nectar traits influence visitor's performance throughout flower lifetime and if honeybees (*Apis mellifera scutellata* Lepeletier, 1836) are effective and efficient for coffee pollination comparing fertilization and fructification among four experimental treatments: open (OP), wind (WP), cross (HCP), and single-visit bee pollination (SVBP). We found that honeybees collect both nectar and pollen from coffee flowers and transfer pollen on stigmas even after one visit. No differences were found among treatments regarding the number of pollen grains transferred on the stigmas (effectiveness). OP flowers showed a comparative lower efficiency (pollen tubes and fruit set) probably due to pollination failure as those flowers have a higher variability on the number of deposited pollen grains. Two of the treatments (HCP and SVBP) showed higher fertilization (measuring tubes until the end of the style). Pollen loads seem to be limited by a peak of pollen transference by pollinators, followed by the stabilization in the number of pollen grains deposited per stigma. Thus, reproduction of the coffee can be limited by the quality of pollen grains moved by pollinators instead of quantity. Management strategies should focus on monitoring bee density on plants for increasing pollen quality transfer on flowers trough maintaining the adequate proportions of seminatural habitats and/or the number of hives on agricultural fields according to the flowering of the crop.

Introduction

Animal pollination is a fundamental step in seed and fruit production for wild and crop plants (Klein *et al* 2007; Ollerton *et al* 2011; Garibaldi *et al* 2011). It is estimated that 78 to 94% of angiosperm species depend on animal pollination to produce fruits and seeds and that 87% of the crops are favored to some degree by the presence of pollinators (Cane & Schiffhauer 2003; Klein *et al* 2007; Altieri 2015). Nevertheless, pollen flowing from the anther to the stigma (i.e. pollination) does not guarantee the subsequent development of seeds and fruits (Proctor *et al* 1996, Ne'eman *et al* 2010) because this process depends on both the quality and quantity of the pollen load (Petit *et al* 2009). More effective pollinators will be those that can move large quantities of conspecific pollen (Aizen & Harder 2007). In this sense,

knowledge on how pollinators affect pollen deposition, fruit set, and fruit quality is essential to understand the pollination requirements of different crops and to develop agricultural practices favoring effective pollinator species which could help to reduce pollination-dependent yield deficits (i.e., inadequate pollen receipt that limits agricultural output) (Vaissière *et al* 2011).

Coffea arabica L. (Gentianales, Rubiaceae) is a crop of global importance and cultivated in approximately 80 tropical countries, of which Brazil is the largest producer (Schmitt 2006; ICO - International Coffee Organization 2012). Although Arabica coffee is an autogamous species, several studies report that the presence of pollinators can increase yield from 10 to 50% (Klein *et al* 2003a, b, c; De Marco & Coelho 2004; Ricketts *et al* 2004; Vergara & Badano 2009; Hipólito *et al* 2018). Non-native honeybees, *Apis mellifera* Linnaeus, 1758, could be the most frequent pollinator on coffee plantations (Roubik 2002), including those from Brazil (Hipólito *et al* 2018).

Despite the large number of studies on coffee pollination, few papers have investigated aspects of floral biology in conjunction with pollinators and their effectiveness and efficiency on pollen transfer and fruit formation (as revised by Ngo *et al* 2011). Most studies on coffee used indirect methods to test pollination (open versus closed flowers) and then correlate this reproductive parameter with floral visitors (Saturni *et al* 2016; Hipólito *et al* 2018) or with a single bee visit (e.g Klein *et al* 2003a). These previous studies on coffee also inferred linear trends between pollination and plant reproductive performance, generating some uncertainties in the interpretation of the results. For example, the saturating relation between pollination and crop production, mask not only the temporal instability of the pollination service but also the yield variations (Garibaldi *et al* 2011).

The evaluation of pollinator performance can be measured at different stages of the pollination and fructification processes and through different indicators related to the plant-visitor relationship. For example, most frequent metrics are those related to the (i) number of visits per flower per unit time (frequency), the (ii) pollinator success in the transference of pollen (pollen deposition effectiveness; "effectiveness" from now on), and/or (iii) the pollinator contribution to the female reproductive success (efficiency) (Ne'eman *et al* 2010).

Pollinator performance can be affected by variables related to pollinator preferences on a specific plant (Totland & Matthews 1998) because particulates in the floral biology and the resources presented as rewards for floral visitors (Prasifka *et al* 2018; Vandelook *et al* 2019). Among floral resources, nectar has been recognized as the most important influencing pollinator preferences (Galetto & Bernadello 2005). As nectar can vary through the floral life cycle (regarding quantity, sugar concentration, and chemical composition) its investigation may help to better understand the constancy and preferences of pollinators on some flowers or species (Galetto & Bernadello 2005; Prasifka *et al* 2018). Nogueira-Neto and Antunes Filho (1959) observed that differences in floral stages on coffee flowers (old and new flowers) implied differences in the behavior of pollinators; however, no details are provided. As far as we know, there are no studies on coffee relating nectar traits for different floral stages with pollinators and pollination.

In that manner, the aim of this study was to analyze nectar traits through flower lifetime and relate these data with visitors' performance, effectiveness, and efficiency. To reach this objective we analyzed if (1) nectar traits were different between floral stages (new and old flowers) and if the variations in nectar traits through flower lifetime affect visitor's performance (per flower frequency of visits and per flower standing time). As the Africanized honeybee is by far the most common floral visitor in coffee flowers, we specifically tested (2) its effectiveness after one single visit (i.e., pollen transference to the stigma) and its efficiency by measuring plant reproductive performance (i.e., pollen tube growth, formed fruits, and fruit quality; Fig 1).

Material and Methods

Study region

The study was performed on a coffee farm called "Fazenda Mussambé" located in the municipality of Ibicoara at the region of Chapada Diamantina, Bahia state, Brazil (13°19.327'S, 41°19.348'W) during two periods: (i) October 2012 (honeybee and floral resource sampling), and (ii) November 2016 to May 2017 (pollination experiments). The farm is located within a cultivated landscape, in which a 2.5-km ratio is possible to find other crops such as passion flower, tomato, bananas, and strawberry (data from SPOT image, year 2009, 5-m spatial resolution). This area can be divided into natural vegetation (native semi-deciduous forests; 25%), coffee (18%), other crops (52%), and areas with water or buildings (remaining 5%). The climate in this region shows a clearly defined wet season (November to March), a mean annual precipitation of 1379 mm, and mean annual maximum and minimum temperatures of 25.7 and 16°C, respectively (INMET 2013).

Coffee plant is a shrub with gregarious flowering, which means that all plants of a particular species flower at the same time in a given geographic extent (Mendes *et al* 1961). Plants present few blooming periods per year in Brazil and flowers remain open for 3 to 5 days (De Castro & Marraccini 2006).

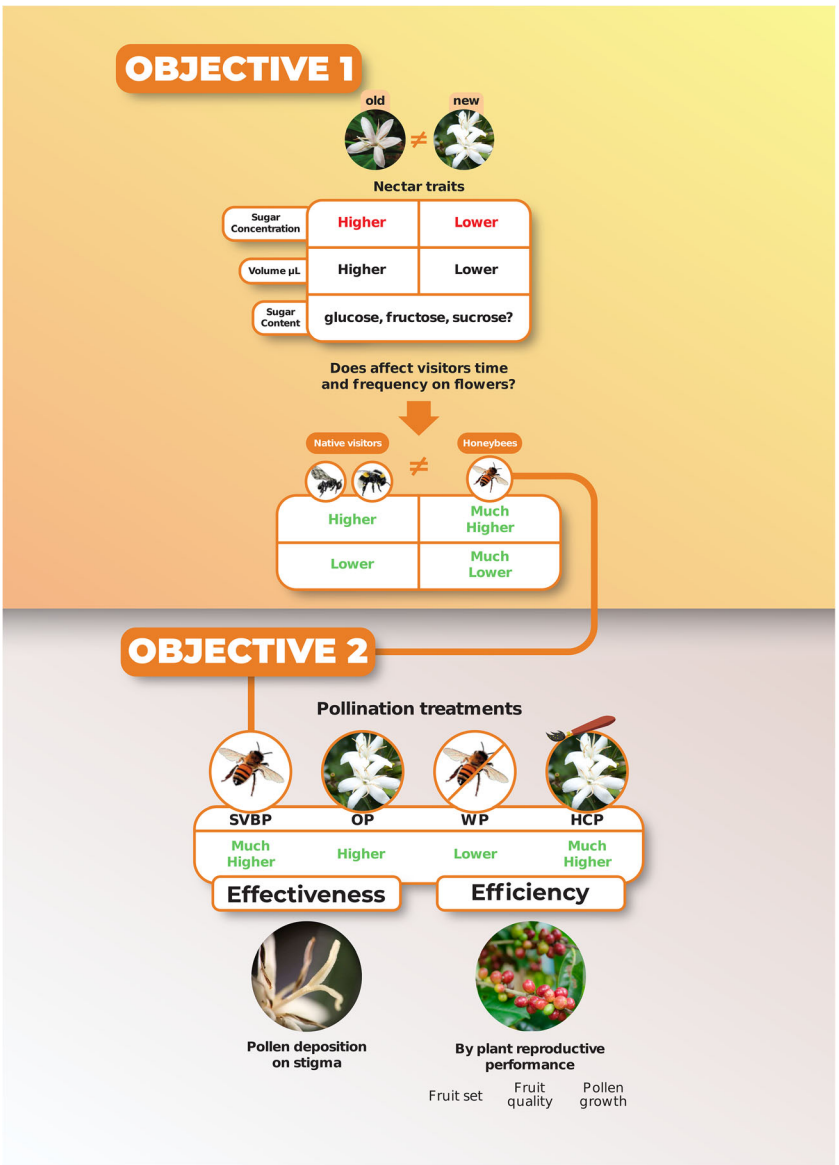

Fig 1 Diagram scheme representing the structure of this study: Objective 1 analyze if nectar traits were different on floral stages (new and old flowers) and if those differences influenced visitor's performance on the Africanized honeybee, *Apis mellifera scutellata*, and other floral visitors. Objective 2 test honeybee effectiveness after one single visit (SVBP) (i.e., pollen transference to the stigma) and its efficiency by measuring plant reproductive performance (i.e., pollen tube growth, formed fruits, and fruit quality) and then comparing results of the SVBP treatment with flowers that remained open-pollinated (OP) and wind-pollinated (WP) or were hand cross pollinated (HCP)

Nectar traits and visitor's performance

Fifteen flowers in different floral stages (with new, $n = 8$ and old anthers, $n = 7$) were gathered and nectar volume was measured with microcapillary tubes of 1–5 µL; sugar concentration was measured using a pocket refractometer (0–30% BRIX). To obtain the total sugar content per flower we used volume and concentration data using the average amount of sugar produced per flower expressed in milligrams following Galetto & Bernadello (2005). Nectar produced by flowers (five samples: three from recently open flowers and two from older flowers) were collected in the field with glass capillary tubes and stored at low temperature on Whatman® Number 1 paper filter (Sigma-Aldrich Co.) until the biochemical analysis. The stored nectar was dissolved in distilled water before sugar composition analysis by spectrophotometry. For the quantitative sugar composition analysis, reagents kits for glucose, fructose, and sucrose (Sigma-Aldrich Co., St. Louis, MI, USA) were used following the methodologies proposed by Bergmeyer & Bernt (1974) and Southgate (1976). The absorbance reading was determined at a wavelength of 340 nm in a spectrophotometer (Metrolab 330, Switzerland). The proportion of sugars was expressed as means ± standard deviation.

In order to evaluate visitor's performance (frequency of visits and per flower standing time) on coffee flowers, three transects of flowering plants within 3-m^2 quadrats were visually defined. On these sampling units, flower visitors were continuously observed from 0900 to 1500 h, during two consecutive days. Flower visitors touching anthers and

stigma were considered as pollinators. The time period that a pollinator spent on individual flowers, flower stage, and the resources collected during their visits (nectar and/or pollen) were recorded. Insect species were identified by simple observation to the lowest possible taxonomic level.

Pollination effectiveness and efficiency

In order to evaluate effectiveness and efficiency of flower-visiting insects we performed pollination experiments with four treatments: open pollination (OP), wind pollination (WP), hand cross pollination (HCP), and single-visit bee pollination (SVBP) by the subspecies of *Apis mellifera* present in the study area (Kerr 1967; Sheppard *et al* 1991) the Africanized honeybee (honeybee from now on), as described below. These experiments were conducted between November 2016 and May 2017. We established a 25 × 50 m plot, considering seven rows where we chose plants and flower buds to be analyzed according to four pollination treatments: (a) OP*:* flowers were exposed to flower visitors during the entire flower lifetime; (b) WP*:* buds were covered with voile bags (0.5-mm mesh; handmade by a seamstress) to exclude insect visitors but theoretically allowing the airflow with pollen grains (as pollen grains size are approximately 0.03 mm); these flowers remained bagged during their entire lifetime; (c) HCP*:* bagged flowers with voile were manually pollinated with a brush using pollen collected from flowers of at least three different neighboring plants; (d) SVBP: previously covered flowers were exposed to pollinators by removing voile bags; when an individual flower received a single honeybee visit it was marked and bagged again to avoid further visits. Flowers of each of the four treatments were collected to quantify pollen deposition (to test effectiveness and efficiency; see below) or remain tagged in the plants until fruit maturation (to test efficiency and fruit quality; see below).

Pollination effectiveness

Pollination effectiveness was evaluated by counting the total number of pollen grains on stigmas of the different pollination treatments that were collected after anthesis (OP, $n = 49$; WP, $n = 40$; HCP, $n = 12$; SVBP, $n = 17$). To compare pollination effectiveness between pollination treatments, the total number of pollen grains deposited on stigmas during flower lifetime (3 days) were counted under an optic microscope.

Pollination efficiency and plant reproductive performance

Pollination efficiency was evaluated by measuring plant reproductive performance (i.e., pollen tube growth, formed fruits and fruit quality) among pollination treatments. To compare plant reproductive performance among pollination treatments, we collected flowers pistils (OP $n = 49$, WP $n = 40$, HCP $n = 12$, and SVBP $n = 17$) and stored them separately in tubes (Eppendorf's 15 mL) with FAA (50 ml of 70% alcohol, 50 ml of acetic acid, and 900 ml of formaldehyde). In the laboratory, the pistils were screened and passed through the washing procedure adapted from Kho & Baer (1968), immersed in aniline blue dissolved in potassium acetate (0.1%) during 2 h, and then mounted in slides to be analyzed by fluorescence microscopy. For each pistil, three data were registered: the number of deposited pollen grains, the number of pollen tubes developed at the top of the style, and the number at the top of the ovary.

Fruit set was registered on tagged flowers of the pollination experiments (OP, $n = 49$; WP, $n = 41$; HCP, $n = 12$; SVBP, $n = 17$) that remained on plants during the whole reproductive period until matured fruits were collected (final fruit formation after 6 months). Fruit size and weight were used as proxies to compare fruit quality among treatments. Matured fruits were measured with a digital caliper in order to obtain fruit height and width (fruit size). In addition, fruit mass was obtained with a precision digital balance (Ohaus Adventurer - AR2140).

Data analysis

To assess the relationship between *effectiveness and efficiency*, we used a generalized linear model (glm) where "pollen grains" (number) and fruit set were the dependent variables. Pollination treatments (OP, WP, HCP, and SVBP) were used as the explanatory variables. As pollen grains presented a negative binomial distribution and fruit set a binomial (formed = 1, or aborted = 0) we used the function glmmTMB in R software.

Complementary to analyzing the effects of pollination efficiency and plant reproductive performance, we used a generalized linear model (glm), where pollen tube growth and fruit quality were considered as dependent variables. We used the beta probability distribution for variables related to pollen tube growth (initial growing at the style top and final growing at the ovary top) and the Gaussian distribution for fruit variables (height or size and mass). For the fruit set, we used a binomial distribution. The number of pollen grains was used as a predictor variable with a quadratic term. We assume that the effect of the number of pollen grains deposited on the stigma reaches an asymptote for the number of pollen tubes growing in the style, as well as for fruit quantity and quality. This allowed us to investigate the pollen deposition curve (or saturation effect), which cannot be analyzed if one assumes a linear positive pattern between these variables. For fruit variables (fruit quality), we considered only the OP (open pollination) and (WP) wind pollination treatments because sample sizes from HCP and SVBP treatments

were too low (< 20) for curve estimations. All analyses were performed in R software version 3.5.0 (R Core Team 2018).

Results

Nectar traits and visitor's performance

Nectar measurements showed that new flowers held more nectar (3.9 ± 2.2 μL; ca. 1 mg of solutes) than older flowers (2 ± 0.5 μL; ca. 0.5 mg of solutes). Sugar concentration was relatively constant (with values from 22 to 25% BRIX) during flower lifetime. Nectar sugar composition was almost invariable during flower lifetime: glucose (new flowers = 19.6 ± 1.3%; old = 19.5 ± 1.4%), fructose (new flowers = 30.9 ± 1.8%; old = 32.3 ± 0.2%), and sucrose (new flowers = 49.5 ± 2.5%; old = 48.3 ± 1.7%).

In a total, we were able to observe 242 events in which different insect species visited individual coffee flowers. Honeybees were the most abundant flower visitor on coffee flowers (78.4%). Other visitors included ants, butterflies, flies, and wasps (17%) as well as other bees (*Bombus, Trigona*, and other stingless bees) (4.4%). Most *Trigona* bees and ants performed illegitimate visits, perforating floral structures and not contacting the reproductive structures of the flowers. Most legitimate flower visitors, including honeybees, were observed gathering nectar (70.6%) (Table 1). We also registered some visitors collecting just pollen, or nectar and pollen together, as well as visitors avoiding flowers previously visited by other insects. Many insects did not land on flowers and only occasionally touched flower structures but this data set was not included in the analyses (Table 1). In general, most visits were observed on new flowers (238); in consequence, we did not split our data set between visits to old and new flowers.

Honeybees were the most frequent flower visitor and we have collected enough data to compare their behavior regarding their time spent on flowers when gathering floral resources. We observed that when honeybees are gathering both pollen and nectar from the same flower, they spent more time (18.9 ± 16.7 s) than when they collect only nectar (3.9 ± 2.7 s) or pollen (7.9 ± 6.7 s) (Table 2).

Table 1 Resources collected (in percentages) by the different groups of flower visitors on coffee flowers. The values related to resources collected were obtained from the sum of the number of insects counted, acquiring different resources (nectar and/or pollen) and transformed into percentages. The *Trigona* bees and ants mostly made illegitimate visits and were therefore excluded from the comparison

Group of flower visitors	Resource collected in %		
	Nectar	Pollen	Nectar and pollen
Honeybees	51.4	15	8.9
Bombus	2.8	0	0
Meliponini bees	0.9	0.9	0.5
Other visitors	15.4	4.2	0

Pollination effectiveness and efficiency

Pollination effectiveness (mean number of pollen grains deposited on stigma) did not show differences among treatments ($p = 0.782$). The OP treatment has presented the higher variability in the number of pollen grains deposited per stigma (Fig 2a). On the contrary, pollination efficiency (mean plant reproductive performance i.e., pollen tube growth, formed fruits, and fruit quality) showed differences among treatments ($p = 0.014$). The OP treatment produced a slightly lower percentage of formed fruits (fruit set) than the other pollination treatments ($p = 0.058$) (Fig 2b).

Pollen tubes and fruit quality were influenced by different variables (Table 3). We observed that the addition of pollen grains increases the number of tubes in the pistil (both at the top of the style and at the top of the ovary) (the linear term was positive) but reached a maximum (or a limit) (the quadratic term was negative) (Table 3). When we observed the number of pollen tubes formed on the top of the style we found that the OP treatment presented the higher values than other treatments (Fig 3a). However, this trend is not maintained for pollen tubes at the ovary top because the HCP treatment presented the highest values followed by those obtained for the SVBP treatment (Fig 3b). For fruit quality measures, OP treatment (compared with WP only) showed the higher values for fruit size (Fig 4).

Discussion

This study demonstrated that honeybees spend enough time on flowers and transfer sufficient pollen grains after one visit to guarantee pollen tubes reaching the ovary to fertilize the ovules (realized pollination). The high abundance and frequency of honeybees on coffee flowers has been reported previously (Ricketts *et al* 2004; Vergara & Badano 2009; Bravo-Monroy *et al* 2015), including studies performed in the same region as the present work (Hipólito *et al* 2018). The resource collected and time spent on a flower by a floral visitor can affect pollination success (Cane & Schiffhauer 2003; Monzón *et al* 2004). In our study, most floral visitors including honeybees were observed gathering only nectar (61.3%), and less than 26% were observe collecting only pollen, or both resources concomitantly.

As far we know, this is the first study to effectively test honeybee pollination on coffee linking pollen quantity/quality measures to fruit maturation and fruit quality and

Table 2 Honeybee's time on flowers (in seconds) during a single visit in order to obtain nectar, pollen or both resources on flowers of coffee. Values represent mean and standard deviation (SD) as well as maximun and minimum values found per visit considering each of the resource

Resource	Mean ± SD (seconds per visit)	Maximum value (seconds per visit)	Minimum value (seconds per visit)
Nectar	3.9 ± 2.7	13.1	0.8
Pollen	7.9 ± 6.7	27.29	1
Nectar and pollen	18.9 ± 16.7	60	2

not inferring these relationships only through (i) visual observations (e.g., Nogueira-Neto & Antunes Filho 1959; Amaral 1952; Corbet 1987) (ii) or by comparing open flowers (available to pollinators) versus other treatments (flowers with hand pollen supplemented and/or bagged flowers) with no specific control for the number of visits and pollinator identity on flowers (e.g. Klein *et al* 2003a, b, c; Vergara *et al* 2008; Krishnan *et al* 2012; Hipólito *et al* 2018).

Distinctive behaviors can be found within different *Apis mellifera* subspecies, whose genetic composition of the colonies may vary according to climate variables (Abou-Shaara 2014). Our data indicate that the subspecies *A. mellifera scutellata* could be an important pollinator for coffee in Chapada Diamantina, Bahia, Brazil, as with only a single visit this subspecies produces comparable fructification levels than the hand cross supplemented treatment. However, we do not know the effects of repeated visits to the same flower by honeybees on the fruit set, because increasing the number of visits to the flower would determine a subsequent decrease in the pollen load and/or stigmatic damages (Sáez *et al* 2014).

Pollination effectiveness (i.e., the mean number of pollen grains deposited on the stigma) did not differ statistically between pollination treatments. The higher variability in pollen deposition on stigmas observed in the open pollination (OP) treatment could be explained by the fluctuating frequency of visitation per flower and/or by varying amounts of self-pollen deposition. Nevertheless, the number of samples gathered in this study could be low to detect differences among pollination treatments. In consequence, although we found some interesting trends, new studies increasing samples sized are recommended.

Coffee flowers from the hand cross pollination (HCP) treatment showed a higher number of pollen tubes growing in the upper part of the style and a slightly higher fruit set. This trend may suggest an improvement in the crop yield through the increase of pollen loads but also of pollen quality. Previously published results in coffee evidenced a higher fruit set in open-pollinated flowers compared with bagged flowers (from 10.5 to 50%) (Badilla & Ramirez 1991; Raw & Free 1977; Roubik 2002; Klein *et al* 2003a, b, c; Bravo-Monroy *et al* 2015; Saturni *et al* 2016; Hipólito *et al* 2018). However, most of those studies only evaluated fruit set comparing exposed versus closed flowers and not pollination effectiveness and efficiency). Here we did not find differences in fruit set comparing hand cross treatment and bagged flowers, but some differences emerge when fruit quality was considered. The self-pollen deposition could be dominant in the closed pollination treatment (WP) determining the lower fruit quality compare to the open pollination (OP) treatment. Cross-pollen mediated by insects showed the best performance in terms of fruit quality which could be related to a higher genetic pollen quality (Aizen & Harder 2007).

It has been reported higher initial fruit set rates in outcrossed flowers compared with manual self-pollinated

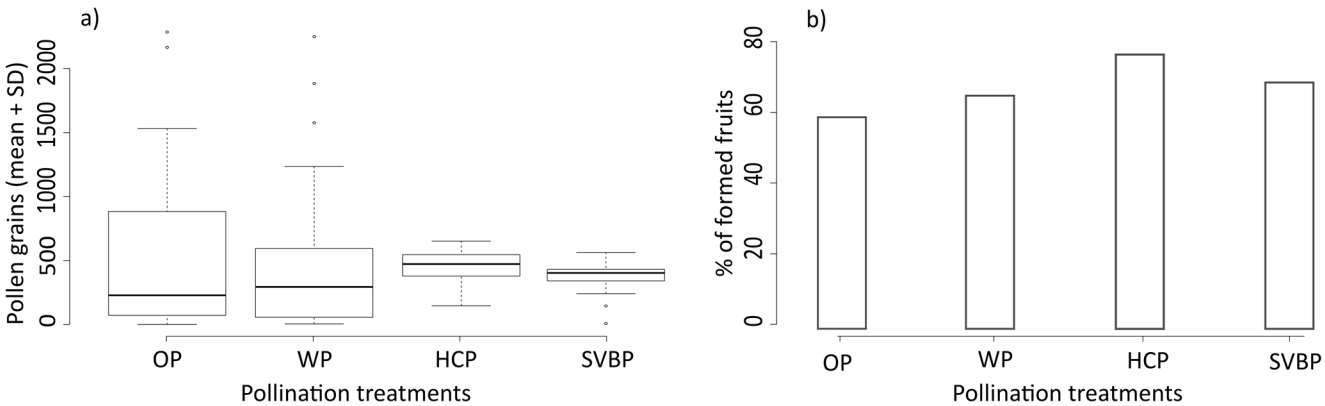

Fig 2 Effectiveness (**a**) and efficiency (**b**) of following pollination treatments performed on coffee flowers: open pollination (OP, $n = 49$); wind pollination (WP, $n = 40$); hand cross pollination (HCP, $n = 12$) and single visit bee pollination (by the Africanized honeybee, *Apis mellifera scutellata*) (SVBP, $n = 17$). Effectiveness = number of pollen grains deposited per stigma. Efficiency = number of fruit sets calculated as the ratio between % of formed fruits/number of tagged flowers. Graphs evidence results from the generalized linear model (glm) with the a) lack of differences on treatments regarding pollination effectiveness and b) a slightly lower percentage of formed fruits on open pollination treatment

Table 3 Fit of the generalized linear model (glm) used to evaluate the effects of pollination efficiency and plant reproductive performance on coffee plants considering pollen tube growth (on the top and on final) and fruit quality (size and weight) as dependent variables vs. the number of pollen grains linear, number of pollen grains quadratic, and pollination treatments. Pollination treatments utilized were open pollination, wind pollination, hand cross pollination, and single-visit bee pollination. Columns represent the evaluated responses (measurement units in brackets) and rows indicate the statistical model components (number of pollen grains and pollination treatments). Only the treatments HCP (hand cross pollination) and OP (open pollination), appeared at the final models. Cells indicated as "–" refer to predictors not selected in the fit model. β ± SE. Samples sizes are presented in Fig 2 and in "Material and Methods"

		Pollen tube growth		Fruit quality	
		On top (number)	On final (number)	Size (mm)	Weight (mg)
Fixed effects	Number of pollen grains (linear)	+ 5.89 ± 0.23	+ 3.32 ± 0.47	+ 4.57 ± 1.42	+ 1.01 ± 0.25
	Number of pollen grains (quadratic)	− 4.37 ± 0.23	− 1.79 ± 0.48	–	–
	Hand cross pollination	− 0.35 ± 0.11	+ 0.31 ± 0.16	–	–
	Open pollination	+ 0.34 ± 0.07	+ 0.62 ± 0.36	–	–

flowers in *C. arabica* (Klein *et al* 2003a), indicating that not just quantity but the quality of pollen are important factors in the pollination of this self-compatible species (Rickets 2003). There is a general agreement that the amount of cross-pollen reaching the coffee stigma is more important than the total number of pollen grains, Peters & Carroll 2012) since coffee has only two or sometimes three ovules.

The relationship between the number of pollen grains and the number of pollen tubes that grew at the top and the base of the style indicates there is an increase in the number of pollen tubes until they reach a peak and thereafter the number decreases. As pollen loads are increased, the number of growing pollen tubes could be clogged within the style, interfering in their development to reach the ovules (Young & Young 1992).

Pollination efficiency and fruit quality could also be affected by post-pollination processes not considered here as pollen tube competition, resource allocation, genetic compatibility systems, water, and nutrients supply and crop management (Pías & Guitián 2006; Burd 2008; Vaissière *et al* 2011; Klein *et al* 2015; Garibaldi *et al* 2011). Yet, relationships between pollinator behavior and pollen quality deposition may represent trade-offs between the benefits of increased pollinator

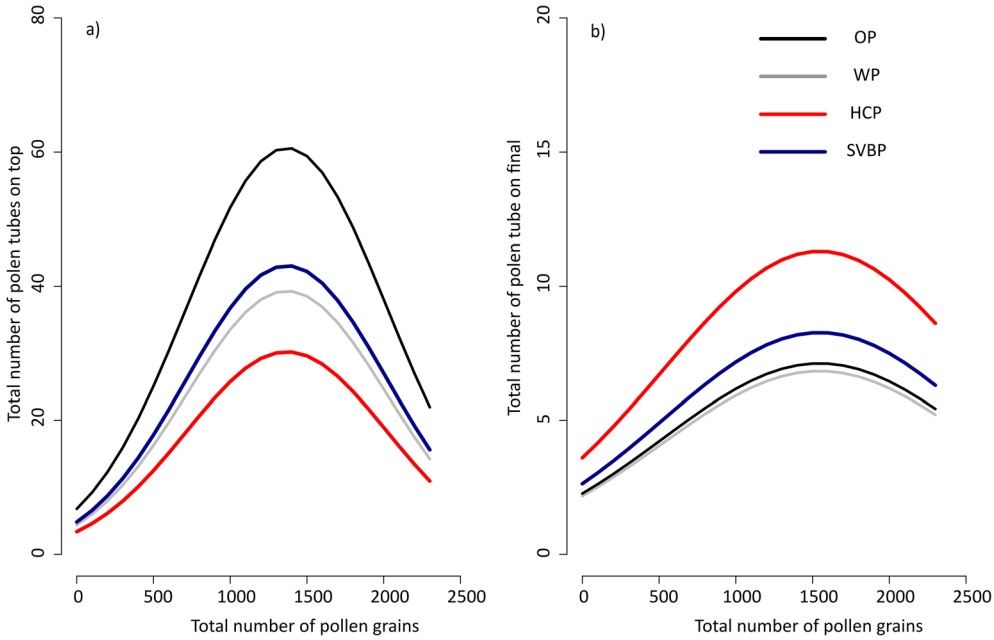

Fig 3 Relationships between the total number of pollen grains per stigma and the number of pollen tubes growing on the upper part (**a**) and on the final part of the style (**b**) of coffee flowers found of results of the following pollination treatments: open pollination (OP), wind pollination (WP), hand cross pollination (HCP), and single-visit bee pollination (by the Africanized honeybee, *Apis mellifera scutellate*) (SVBP). Samples sizes can be found in Fig 2 and in "Material and Methods." Curves represented by different treatments evidences differences on results when comparing a) the number of pollen tubes on the upper part of the style (top) and on b) final. While open flowers (black curves) have more pollen tubes on top of styles, single-visit bee pollination (red curves) has more pollen tubes at the end of styles

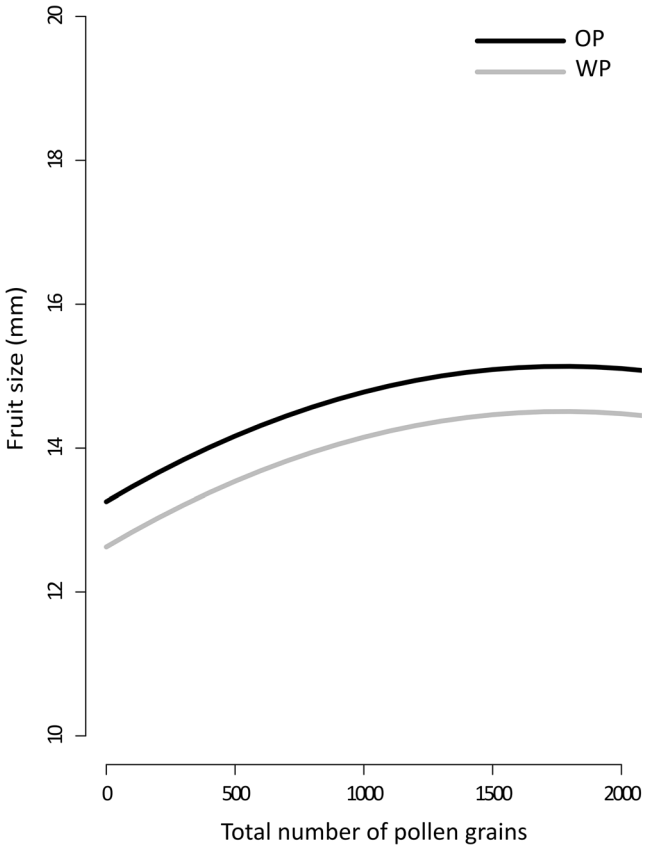

Fig 4 Relationship between the total number of pollen grains deposited on the flower stigma and the final coffee fruit size (mm) by open (OP) and wind (WP) pollination treatments (see sample sizes in Fig 2 and in "Material and Methods"). Curves represented by different treatments from the generalized linear model (glm) evidence that fruits from open flowers (i.e., exposed to flower visitors) generate slightly bigger fruits

visitation and the quantity/quality of pollen received and the reproductive costs of increased self-pollination and reduced pollination quality and fruit set.

Summarizing, a higher number of pollen grains on the stigma can be translated into higher fruit quality (size and weight) but with some trade-offs as the balance of self- and cross-pollen or the number of pollen tubes competing for ovule fertilization. Peters and Carroll (2012) found that pollen deposition and initial fruit set rates were not correlated in *C. arabica*, probably because this is not a linear relationship (Aizen & Harder 2007), where stigmatic pollen loads may not translate directly and linearly into fruit quality (i.e., fruit size or viable seed counts) (Cane & Schiffhauer 2003, our results). Although flowers from the open pollination treatment showed a lower proportion of formed fruits than the autogamous treatment (bagged flowers), the fruit quality was improved. Comparable results were obtained elsewhere for *C. arabica* (Philpott *et al* 2006; Classen *et al* 2014), which support our findings.

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