

## Analysis of the effects of climate variables on *Apis mellifera* pollen foraging performance

[Análise dos efeitos de variáveis climáticas no desempenho de forrageamento de pólen de abelhas *Apis mellifera*]

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### ABSTRACT

Pollination services performed by bees are essential for the reproduction of a great part of flowering plants. The pollen collected by *Apis mellifera* while performing pollination (bee pollen), has been incorporated into the human diet for its favorable nutritional components. Around 1,500 tons of bee pollen are produced annually worldwide, especially in Spain, China, Australia, Argentina, and Brazil. Despite the importance of bee pollen within apiculture, little is known about the effects of climate variations on bee pollen collection and production. We monitored the pollen collection performance of 24 different honey bee colonies in different climate conditions within a period of one year. We then analyzed the statistical interaction among the number of worker bees returning with pollen loads and 12 climatic variables, to produce a predictive mixed linear model. The results obtained showed that 7 climatic variables were statistically correlated to the pollen collection observed: Maximum temperature of the day, minimum temperature of the day, dew point temperature, relative humidity, cloud cover, rainfall, and the date of the sample. This research brings information for the development of a more effective pollen productive system.

Keywords: africanized honey bees, bee pollen, climate, foraging, pollination

### RESUMO

Serviços de polinização são essenciais para a reprodução de uma grande parte das plantas com flores. Além de fundamental para produtividade agrícola e segurança alimentar no planeta, a atividade de polinização por *Apis mellifera* também possibilita a produção do pólen apícola. Devido a sua riqueza nutricional, o pólen apícola vem sendo incorporado às dietas humanas e de animais de interesse zootécnico. Cerca de 1.500 toneladas de pólen de abelha são produzidas anualmente em todo o mundo, especialmente em países como Espanha, China, Austrália, Argentina e Brasil. Apesar da importância do pólen na cadeia produtiva apícola, muito pouco se conhece sobre os efeitos das variações climáticas sobre a coleta e a produtividade de pólen. Neste estudo, foi monitorado o desempenho da coleta de pólen de 24 colônias de abelhas africanizadas, em diferentes condições climáticas, por um período de um ano. Analisaram-se estatisticamente as interações dos dados obtidos para o número de abelhas operárias que retornaram com cargas de pólen nas corbículas e 12 variáveis climáticas, a fim de se produzir um modelo estatístico preditivo. Os resultados obtidos mostraram que sete variáveis climáticas influenciaram significativamente a coleta de pólen: temperatura máxima do dia, temperatura mínima do dia, temperatura do ponto de orvalho, umidade relativa, cobertura de nuvens, precipitação pluviométrica e data da amostragem. Esta pesquisa traz informações importantes para o desenvolvimento de um sistema produtivo de pólen apícola mais eficaz.

Palavras-chave: abelhas africanizadas, pólen apícola, clima, forrageamento, polinização

### INTRODUCTION

Today more than 83 million beehives are kept worldwide (FAOSTAT, 2014). Honey is, for sure, the most consecrated bee product, but it is not the only one, and it is not the most important

(Cvitkovi *et al.*, 2009). Pollination services performed by wild and kept bees are essential for the reproduction of a great part of flowering plants, once pollination promotes a more effective transference of genes among populations of wild and cultivated plant species (Kearns *et al.*, 1998). The importance of insect

pollination has been valued at over €153 billion, annually, for agricultural production (Gallai *et al.*, 2009). The pollen collected and transferred among different flowers (pollination) promotes plant reproduction and, as a mutualistic relationship, provides bees the collected surplus pollen as a nutritional resource for protein, lipids, and antioxidant substances (Campos *et al.*, 2010). For its nutritional richness, the pollen collected by *Apis mellifera* while performing pollination has been incorporated into the human diet (FAO, 2009). Around 1,500 tons of bee pollen are produced annually worldwide, especially in Spain, China, Australia, Argentina, and Brazil (FAO 2009). Despite the importance of bee pollen within apiculture, many aspects of bee pollen production remain without the deserved attention of science (de Mattos *et al.*, 2016). Thus, there is no standard protocol concerning the most suitable techniques to be applied in the bee pollen production system as well as limited knowledge related to the effects of climate variations on bee pollen collection.

Climatic conditions seem to strongly affect insects, once the variations are capable of impacting behavior, physiology and reproductive success of those animals (Brown and Paxton, 2009). The climate is also able to produce significant effects on plant's phenology, including pollen and nectar (Tooke and Battey, 2010). For that reason, this study aimed to analyze the effects of climate on pollen foraging for the better understanding of what kind of climatic variables are influent on bee pollen production. Studies able to obtain information about such effects are an important tool for the development of a more effective bee pollen productive system.

## MATERIAL AND METHODS

The study was conducted at the University of São Paulo (USP) apiary in Ribeirão Preto – SP, Southeastern Brazil (21° 10' 42" S, 47° 48' 24" W, height: 545m). The apiary was settled in an area surrounded by *Eucalyptus sp* abandoned crop and native vegetation. According to the classification system developed by Köppen and Geiger (1928), the region presents a subtropical climate, ranging from humid to sub-humid. The climate of the region is typically defined by two marked seasons: one cool and dry (April to

September) and another hot and rainy (October to March) (Silva *et al.*, 2014).

For 12 consecutive months (January to December 2014) we monitored the pollen foraging activities in 24 colonies kept in standard Langstroth hives. All the studied colonies had approximately the same population size by the beginning of the study (composed by eight brood frames and two food frames, with bees covering 8 of the 10 frames). All colonies were headed by siblings Africanized honey bee queens of the same age. We observed and recorded (using a hand counter) the number of worker bees returning with pollen loads on their corbiculas, during a period of 3 minutes per day, in each one of the tested colonies. All the observations started at 7:30am, from January to December of 2014. We adjusted the observations during the Brazilian daylight saving time to maintain the same starting hour throughout the year. Twelve climatic variables (independent variable) were daily recorded for further statistical analysis (Table 1). The date of the observation was also analyzed as a variable. For that propose, the sampling dates were converted into ordinal numbers by a count up of the days of the year (January 1<sup>st</sup> as the day 1 and December 31<sup>st</sup> as the day 365). The climatic data were obtained in two databases provided by the Brazilian government and the São Paulo State government (respectively: Instituto Nacional de Pesquisas Espaciais – INPE: <http://www.inpe.br/>; and Centro Integrado de Informações Agrometeorológicas – CIIAGRO: <http://www.ciiagro.org.br/>). Both databases registered climatic variables from the nearest meteorological station to the apiary (approximately 5km away).

The statistical modeling of the data concerning pollen collection and climatic variables were done using generalized linear mixed models. The number of worker bees, returning with pollen loads, was used as the dependent variable and the other 12 climatic data as the independent variables. In order to account the response of individual colonies over time, we included a random intercept for each colony and a random slope, as described in Koffler *et al.* (2015). All continuous variables were centered and scaled to aid model convergence. All statistical procedures were done on SAS (2004).

## RESULTS

The number of foragers per colony collecting pollen ranged from 0 to 443 (during the 3 minutes of observation), with a mean of 69.69 (S.D.  $\pm$  56.31). Temperatures varied from 10.90 to 39.00°C, relative humidity varied from 22 to 100%, cloud cover from 0 to 100%, rainfall from 0 to 23mm and wind speed varied from 0 to 5.10m/s (Table 1). The period of more intense pollen foraging activity was during the summer (from December to March); the mean number of foragers registered was 161.68 (S.D.  $\pm$  115.71). During the winter (from June to September) we registered 19.18 ( $\pm$ 16.99) as the mean number of foragers returning with pollen loads. Within the spring (from September to December) and autumn (from March to June), the number of foragers returning with pollen loads remained between 40.89 ( $\pm$ 40.44) to 30.76 ( $\pm$ 18.87), respectively. Statistical analysis showed that the pollen foraging activity in the summer was significantly more intense than in other seasons of the year: ANOVA (D.F.= 3; 846):  $F= 246.80$ ,  $P<0.001$ . Pairwise comparisons showed significant differences among different seasons (95% C.I. of difference): Summer vs Autumn: 115.20 to 146.70,  $P<0.001$ ; Summer vs Winter: 126.80 to 158.20,  $P<0.001$ ; Summer vs Spring: 105.10 to 136.50,  $P<0.001$ . Foraging activity in the winter was significantly less intense than in the spring (-37.45 to -5.97,  $P=0.001$ ). No significant difference was registered when comparing the mean pollen foraging between

spring and autumn (-25.86 to 5.61,  $P=0.432$ ) (Figure 1). The data obtained were also submitted to statistical analysis regarding the interactions among the pollen collection and climate variables, within each season (Table 2).

A mixed linear model was fitted using all the climatic variables tested. Due to the way the model fitted data, the natural logs of the collected pollen was used instead. The results obtained showed that seven variables tested were statistically correlated to the pollen collection observed (ANOVA): Maximum temperature of the day ( $P<0.001$ ), minimum temperature of the day ( $P= 0.006$ ), dew point temperature ( $P= 0.002$ ), relative humidity ( $P= 0.041$ ), cloud cover ( $P<0.001$ ), rainfall ( $P<0.001$ ) and the date of the sample ( $P<0.001$ ) (Table 2; Figure 2). A predictive mathematical equation was also produced compiling all the statistically significant variables tested:

$$\text{Number of worker bees returning with pollen loads} = 46.5886 + (0.6735 \times A) + (0.06861 \times B) + (0.04815 \times C) + (0.02959 \times D) - (0.00381 \times E) - (0.00539 \times F) + (-0.00267 \times G).$$

A: Maximum temperature of the day; B: Minimum temperature of the day; C: Dew point temperature by the time of the observation; D: Rainfall of the day; E: Humidity by the time of the observation; F: Cloud Cover by the time of the observation; G: date of observation.

Table 1. The climatic variables daily registered by this study

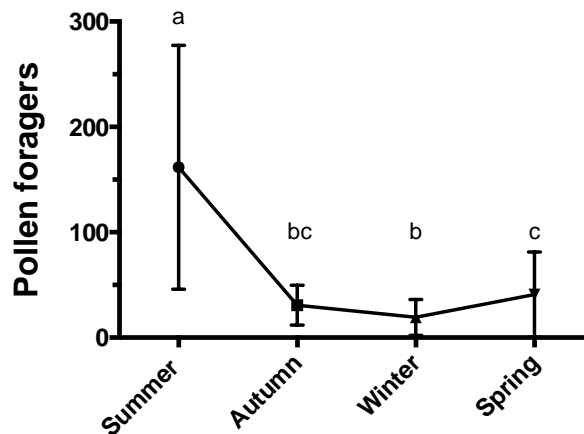
Variables	Measuring unit	Mean	S.D.	Max.	Min.
Pollen foragers	NWB	69.69	56.31	443.00	0.00
Max. temperature	°C	30.52	3.13	39.00	22.40
Min. temperature	°C	17.04	2.730	21.40	10.90
Dew point temperature	°C	15.59	3.45	22.00	6.90
Air temperature	°C	19.93	2.80	35.00	12.30
Relative humidity	%	63.99	31.64	100.00	22.00
Height of the first base of clouds	m	2655.49	339.02	2750.00	75.00
Cloud cover	%	47.69	39.69	100.00	0.00
Rainfall	mm	2.40	1.45	23.00	0.00
Atmospheric pressure	hPa	948.45	7.22	955.50	921.80
Atmospheric pressure variation	hPa	-0.35	0.87	1.70	-1.90
Wind speed	m/s	2.40	1.45	5.10	0.00

NWB: Number of worker bees returning with pollen loads on the corbicula.

Table 2. Multiple regression of the climatic variables affecting pollen collection, within each season

	Summer	Fall	Winter	Spring
Variables (Pearson's correlation index; P value)	MinT (0.316; P<0.001)			
	DewT (0.477; P<0.001)	MinT (0.341; P= 0.002)	MinT (0.341; P= 0.002),	
	MaxT (0.555; P<0.001)	DewT (-0.315; P= 0.004)	DewT (-0.315; P= 0.004)	
	AirT (0.454; P<0.001)	AirT (-0.536; P<0.001)	AirT (-0.536; P<0.001),	MinT (-0.205; P= 0.018)
	AtmV (-0.202; P= 0.001)	AtmV (0.499; P<0.001)	AtmV (0.499; P<0.001)	DewT (-0.200; P= 0.021)
	Rain (0.170; P= 0.005)	AtmP (0.418; P<0.001)	AtmP (0.418; P<0.001)	
	HClO (0.176; P= 0.004),	Humi (0.361; P= 0.001)	Humi (0.361; P= 0.001)	
	CClo (0.241; P<0.001)			
	R <sup>2</sup> = 0.501; R <sup>2</sup> adjusted= 0.476	R <sup>2</sup> = 0.346; R <sup>2</sup> adjusted= 0.305	R <sup>2</sup> = 0.346; R <sup>2</sup> adjusted= 0.305	R <sup>2</sup> = 0.066; R <sup>2</sup> adjusted= 0.047
	P<0.001	P<0.001	P<0.001	P= 0.033

MaxT: Maximum temperature of the day; MinT: Minimum temperature of the day; DewT: Dew point temperature; AirT: Air temperature at the time of the observation; Humi: Relative humidity at the time of the observation; HClO: Height of the first base of clouds at the time of the observation; CClo: Cloud cover at the time of the observation; Rain: Rainfall (24h previous to the time of the observation); AtmP: Atmospheric pressure at the time of the observation; AtmV: Atmospheric pressure variation (three hours previous to the observation time).



ANOVA: F= 246.80; P<0.001

Figure 1. Pollen foraging activity among different seasons. Different letters indicate pairwise significant difference among obtained results. Superior/Inferior bars indicate Standard deviation.

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Table 3. ANOVA table for the linear mixed model and the significance of each climatic variable tested

Variables	Estimate	Standard Error	D.F.	t Value	Pr >  t
Intercept	46.588	11.557	41	4.030	<0.001
Air temperature	0.0231	0.020	622	1.130	0.260
Atmospheric pressure	0.0083	0.006	622	1.310	0.190
Atmospheric pressure var.	-0.058	0.060	622	-0.970	0.330
Cloud cover	-0.005	0.001	622	-4.590	<0.001
Date	-0.002	0.0004	622	-6.070	<0.001
Dew point temperature	0.048	0.016	622	3.010	0.002
Height of first base of clouds	-0.0001	0.0001	622	-1.470	0.142
Humidity	-0.003	0.001	622	-2.050	0.040
Max temperature	0.067	0.018	622	3.630	<0.001
Min temperature	0.068	0.0251	622	2.730	0.006
Rainfall	0.029	0.008	622	3.550	<0.001
Wind speed	0.045	0.032	622	1.420	0.156

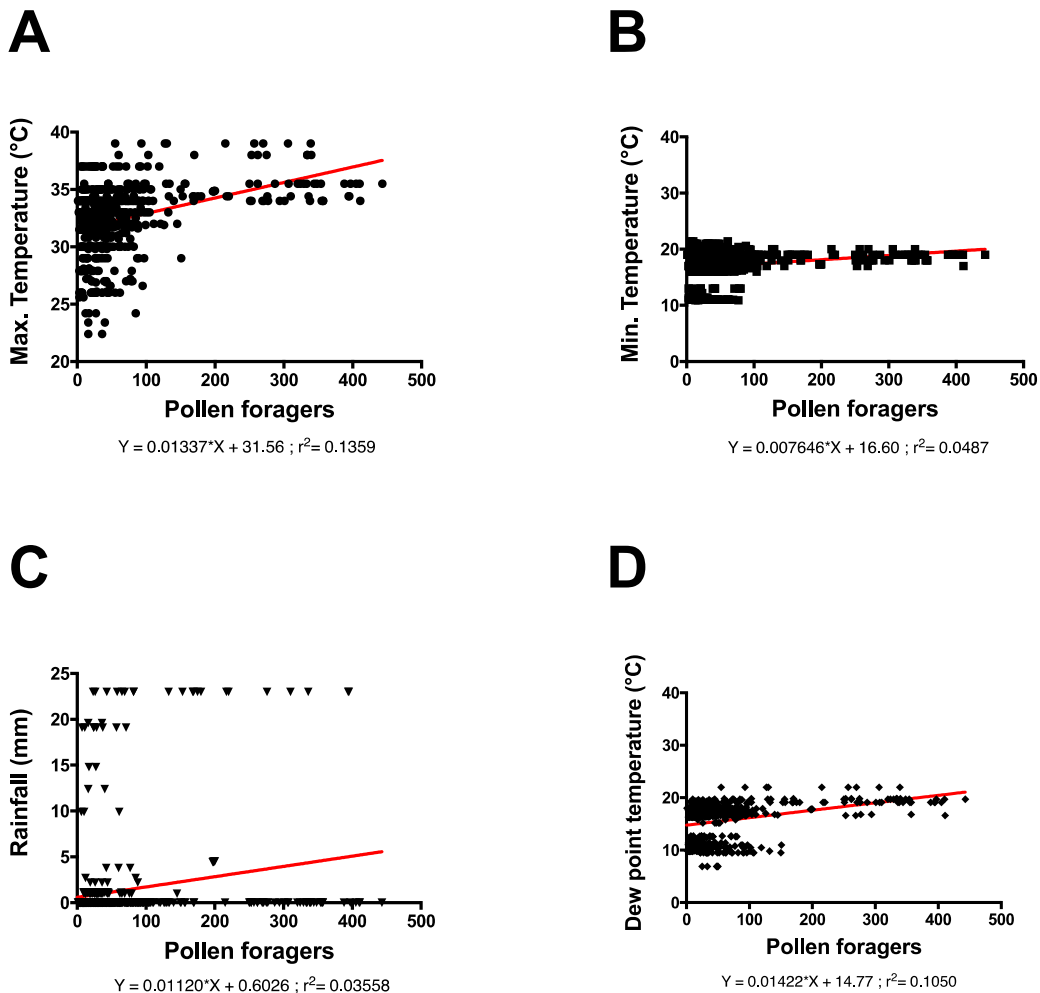


Figure 2. Linear regression between the number of foragers returning with pollen loads and the maximum (A), minimum (B), dew point temperature (C) and rainfall (D).

## DISCUSSION

The observed climate conditions significantly influenced the pollen collection. A total of seven, out of twelve climatic variables presented statistically significant relationship to variations in the number of pollen foragers. The most impacting variables were the ones related to temperature. Several authors observed a similar positive correlation between temperature and flight activity of honey bees (Vicens and Bosch, 2000; Alves et al., 2015). This positive correlation between foraging and temperature can also explain the partial loss of activity/productivity within the winter. Alves et al. (2015) and Malerbo-Souza and Silva (2011) described that the foraging activities of Africanized honey bees usually begins after the temperature reaches a threshold (around 15°C). According to Polatto et al. (2014), African hybrids of *A. mellifera* present more intense activities of flower-visiting and are less susceptible to competition with other bees species, earlier in the day (7:00 to 8:00am). Temperatures below this range may be a limiting factor for the foraging of these bees during the winter. Similar results were obtained by Alves et al. (2015), which observed a more intense foraging activity of Africanized honey bees when the temperature was high (around 30°C) and the relative humidity was low (around 45%). The availability of resources during the winter can also be part of the explanation for the significantly reduced foraging activity.

When the data were analyzed separately (within seasons) we see that the climatic variables present different impact on pollen collection. During the summer, winter and fall, the proposed models could explain a significant fraction of the variability observed in the pollen collection. Although, within the spring the pollen collection seems to be less influenced by the climatic conditions (Table 2).

The surrounding *campus* flora comprises 289 native and introduced species distributed in 232 genera and 73 botanical families, about 67% of those plants present melittophily as the main pollination syndrome (Aleixo et al., 2014; Silva et al., 2014). Among the species present in USP *campus* we can find several specimens of *Alternanthera brasiliana*, *Chamissoa altissima* (Amaranthaceae); *Bidens sulphurea*, *Crepis*

*japonica*, *Montanoa bipinnatifida*, *Sphagneticola trilobata*, *Tithonia diversifolia* (Asteraceae); *Eucalyptus citriodora*, *E. grandis*, *E. moluccana*, *Eugenia brasiliensis*, *E. involucrate*, *E. pyriformis*, *E. uniflora*, *Syzygium cumini*, *S. malaccense* (Myrtaceae); *Paspalum notatum* and *Brachiaria sp* (Poaceae); *Citrus latifolia*, *C. limonia*, *Murraya paniculata* (Rutaceae) (Silva et al., 2014). According to Almeida-Anacleto et al. (2012) species of the Amaranthaceae, Araliaceae, Asteraceae, Myrtaceae, Poaceae and Rutaceae families are important sources of pollen for *A. mellifera* in São Paulo State. Barreto et al. (2006), assert that in the São Paulo State there is more pollen available for bees, in the environment, within periods between January and May, as well as between September and December. This research didn't register the vegetal species producing pollen and the anthesis patterns presented by the flora of the studied area. Although, researches have been showing a flowering peak starting at the transition between the dry and the rainy season (September), in the area (Aleixo et al., 2014). The authors report that the average peak of pollen availability corresponds to February, revealing a seasonal pattern mainly related to the rainy season. Aleixo et al. (2014) also highlight that during the dry season species from the genus *Eucalyptus* and *Eugenia* are an important pollen sources, whereas during the rainy season several species contributed for pollen availability: *Cestrum nocturnum*, *Ludwigia elegans*, *Ricinus communis*, *Bidens sulphurea*, *Dichorisandra thyrsoflora*, *Galinsoga parviflora*, *Sida rhombifolia*, *Solanum violaeifolium*, *Sphagneticola trilobata*, *Tradescantia pallid*, *Chamissoa altissima*, *Cissus verticillata*, *Momordica charantia* and *Solanum seaforthianum* (Aleixo et al., 2014).

In this research, the relative humidity was also related to the foraging activity of the tested bees. This climatic variable was extensively studied and seems to be negatively correlated to foraging (Polatto et al., 2014). Alves et al. (2015) observed that when the humidity was above 81%, there was no foraging activity for Africanized honey bees. It is also considered that high humidity levels can affect sugar concentration in nectar, as well as pollen physical properties (Silva et al., 2013; Alves et al., 2015), thus hampering pollen collection by foragers. Other *Apis* species seems to present the

same patterns of foraging according to the relative humidity intensity. Reddy *et al.* (2015) observed an intense decrease in the activity of *A. cerana* pollen foragers when humidity increased in tropical India. According to the authors, rainfall is closely related to humidity, higher humidity conditions frequently being registered just before, during or just after rainfalls. As rainfall makes foraging activities more difficult and potentially risky, the humidity and the cloud cover tends to present a negative pressure on the flying activity and consequently the pollen foraging.

The results obtained in this research show a low but significant positive relationship between pollen foraging and rainfall. This positive statistical relationship may be explained by the characteristics of the two distinguished seasons found in the region where the data were collected. The dry period (from April to September) coincides with cooler temperature (in which the bee's activity tends to be reduced) and the rainy season coincides with higher temperatures rates and blooming (according to Barreto *et al.*, 2006). It is also important to highlight that the rainfall data used in this research was relative to a period of 24 hours prior to the observation and it is not referent to the exact moment in which the observation was performed.

The cloud cover is generally inversely proportional to solar radiation. We observed a statistically significant negative relationship between cloud cover and pollen foraging, which seems to be in accordance to Vicens and Bosch (2000). The authors observed that *A. mellifera* is fully active at solar radiation higher than 300w/m<sup>2</sup>, being particularly sensitive to drops in solar incidence below this threshold. Further studies are still necessary to evaluate the effects of luminosity on bee pollen production. Moura and Pegoraro (2006); and Polatto *et al.* (2014) also describe a significant influence of sunlight incidence on pollen foraging/production by Africanized honey bees.

Vicens and Bosch (2000) assert that the wind, even at favorable temperatures and light intensity, may cause foraging activity to cease. The results obtained in this research showed a positive relationship of pollen collection and wind speed (0.045) but not statistically

significant (P= 0.156). The narrow range of different wind speeds registered (from 0.0 to 5.1m/s) in the region may be a possible explanation for this lack of statistical consistency. More studies are necessary for a definitive conclusion on the effects of wind speed on pollen foraging.

## CONCLUSION

This research brings information to help the development of a more effective pollen productive system. Our data highlight the significant influence of climate variables on foraging behavior. Thus, beekeepers should be attentive to climate conditions of the regions in which their apiaries are settled to provide suitable conditions for pollen harvesting. We conclude that regions in which temperature range is narrow, rainfall is not scarce (but regularly divided among seasons), the relative humidity is not constantly high and present high solar incidence can be considered more suitable for bee pollen production.

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