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POLLEN FLOW OF *Theobroma cacao* AND ITS RELATIONSHIP WITH CLIMATIC FACTORS IN THE CENTRAL ZONE OF THE ECUADORIAN LITTORAL

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ABSTRACT

Ecuador is known worldwide as a major producer of *Theobroma cacao* "fine aroma", although yields per hectare are in general low. It is one of the most studied tropical species, but there is little scientific information to identify the elements that cause this low productivity which could be related to an inefficient pollen flow. The aim of this research was to quantify pollen flow in clones of *Theobroma cacao* and to assess which climatic factors condition the arrival of pollen to stigmas. The study was carried out in Quevedo, Los Ríos Province, in the Ecuadorian Littoral with a tropical climate. Flowers of different clones were collected for six months. At the beginning of the rainy season (February) the highest pollen flow was observed, with differences between clones varying from 9 to 25 grains per flower, being the clones of Trinitarian origin where the highest arrival of pollen was found, while in the National type clones the lowest reception of pollen grains per flower was recorded. Only 12% of flowers analyzed received more than 25 grains per flower. This flow of pollen is insufficient to withstand pod formation in the cacao trees, which is reflected in the low percentage of tied fruits (effective pollination). The quantified pollen on stigmas is partially determined by the maximum temperature (r=0.46), with minor effects of precipitation (r=0.33), wind speed (r=0.29) and relative humidity (r = 0.27).
Additional keywords: Effective pollination, Malvaceae, reproduction, viability

RESUMEN

Flujo de polen de *Theobroma cacao* y su relación con factores climáticos en la zona central del Litoral Ecuatoriano
Ecuador es conocido mundialmente como uno de los principales productores de cacao "fino y de aroma", aunque los rendimientos por hectárea son en general bajos. A pesar de que es una de las especies tropicales más estudiadas, existe poca información científica para identificar los elementos que causan esta baja productividad, los cuales podrían estar relacionados con un ineficiente flujo de polen. El objetivo de esta investigación fue cuantificar el flujo de polen en clones de cacao y evaluar qué factores climáticos condicionan la llegada del polen a los estigmas. El estudio se realizó en Quevedo, provincia de Los Ríos, en el Litoral ecuatoriano con clima tropical. Se recolectaron flores de diferentes clones durante seis meses. Al comienzo de la temporada de lluvias (febrero) se observó el mayor flujo de polen, con una diferencia entre los clones que varió de 9 a 25 granos por flor, siendo los clones de origen trinitario donde se encontró la mayor llegada de polen, mientras que en los clones de tipo nacional se registró la recepción más baja. Solo el 12 % de las flores analizadas recibieron más de 25 granos por flor. Este flujo de polen es insuficiente para la formación de mazorcas de cacao, lo que se refleja en el bajo porcentaje de frutos (polinización efectiva). El polen cuantificado sobre los estigmas está parcialmente determinado por la temperatura máxima (r=0,46), y en menor grado por la precipitación (r=0,33), la velocidad del viento (r=0,29) y la humedad relativa (r=0,27).
Palabras clave adicionales: Malvaceae, polinización efectiva, reproducción, viabilidad

INTRODUCTION

Theobroma cacao L. is a species native to South America belonging to the Malvaceae family, adapted very well to tropical and subtropical climates (Alverson et al., 1999). *T.*

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cacao is a heterozygous plant with a high variability for agronomic traits and quality (Cope, 1962). It is preferably allogamous, although present studies have shown that many Ecuadorian national genotypes can be auto-allogamous. Generally, it is presumed to be an exogamic species because of its floral morphology and existence of a system of self-incompatibility in some specific genotypes (De la Cruz and Soria, 1973; Falque et al., 1995). Ecuador is known to be the country with the highest production of fine aroma cacao. Nevertheless, fine cacao trees are grown in low input small or medium traditional farms with yields ranking around 7 quintals per hectare. Low productions during the last years have been reported by 900 farmers associated to the Corporación Fortaleza del Valle. Therefore, there is an urgent need to understand the mechanisms of reproduction of cacao, as the key factor underlying new strategies for improving yield as well as the life quality of farmers and their families relaying on cacao for their subsistence.

The reproductive efficiency in *T. cacao* is dependent on both genetic factors (genes that control auto incompatibility, sexual reproduction and reproductive maturity system) and environmental factors (those that alter phenology of flowering and behavior of pollinators) (Banuet et al., 1997). The ecological prediction of reproductive successes of plants and the population dynamics of pollinators implies consideration of flower visitors independently from reproductive system of different genotypes of *T. cacao*, since a drastic specialization could occur in some pollination systems (Waser et al., 2011) . It should be noted that most insects visit flowers with the sole purpose of collecting pollen or other floral rewards, thus preventing those true pollinators to take these same benefits (Inouye, 1980).

Several studies have shown that cocoa flowers are androgynous and are mainly fertilized by insects of the genus *Forcipomyia* of the family Ceratopogonidae (Ollerton et al., 2011; Adjaloo and Oduro, 2013; Chumacero de Schawe et al., 2018). Each floral pillow can produce up to 125,000 flowers per year. Each flower can produce approximately 14,000 pollen grains and up to 74 ovules per ovary (Lopes et al., 2011). However, the rate of natural fertilization (fruit set) of the species reaches only 4 % at the peak of flowering season which corresponds to only an average capture rate of 0.3 insect per flower, thus revealing the low population of effective pollinators for the species (Chumacero et al., 2018).

The great genetic variability of *T. cacao* depends on gene flow by pollen dispersion, which is often substantial among its populations (Ellstrand, 1992). However, the genetic isolation of small populations can also lead to depletion of their genetic variation by drift, because pollen tends to disperse very close to the source. On the other hand, the frequency of pollinations decreases rapidly with distance, where biased leptokurtic scattering curves derived from studies that measure pollen dispersion from a source, become almost axiomatic (Thomas et al., 2009).

Gene flow can vary considerably within species. Studies on pollen flow have shown that within a species dispersion curves can vary considerably with plant genotype (Tonsor, 1985), with vector (Liu et al., 2013), with density of plants (Levin and Kerster, 1969), and during a season (Lachenaud et al., 2007). It directly affects the productive potential of cocoa farms and therefore the economy of producers (Mena and García, 2014).

The aim of this research was to quantify pollen flow (viable and nonviable) in some different clones of *T. cacao* for six months and evaluate which climatic factors condition the arrival of pollen to stigmas. In this sense, gene flow can be an important force in the genetics of plant conservation, and its potential function should be considered in any plant conservation management program (Elistrand, 1992).

MATERIALS AND METHODS

The study was carried out in the experimental farm La Represa, in 2015, owned by the State Technical University of Quevedo, located at 7.5 km of Quevedo-San Carlos road, Province of Los Ríos, Ecuador. The farm is located in an area of flat topography with light slopes and a texture of loamy clay soil. This area is known as a tropical climate. During the season that the study was carried out the daily temperature ranged between 22 and 30º C, monthly rainfall was between 0 and 450 mm (Figure 1) and relative humidity between 80 and 87 % (INAMHI, 2015).

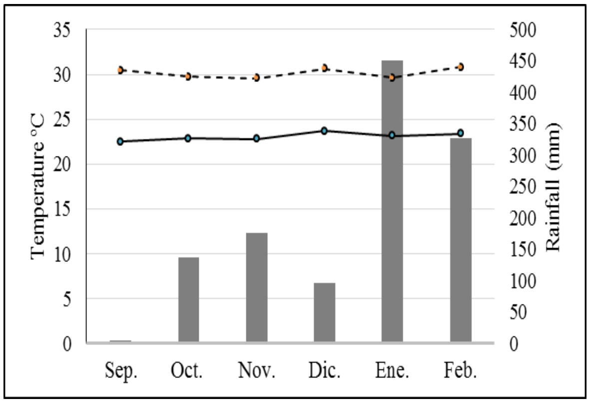

Figure 1. Precipitation (bars), and minimum and maximum temperatures (lines), during the study period in the experimental farm La Represa, Province of Los Ríos, Ecuador

The study was carried out on seven clones of *T. cacao* that are part of a trial of 152 clones selected for their outstanding production characteristics among 6000 trees from seeds established in the experimental farm La Buseta (Sánchez et al., 2013). They were a progeny of the collection "Centro de Cacao Fino and of Aroma Tenguel (CCAT)" in the province of Guayas, with superb production attributes. Among the studied clones there are five of National type (DICYT-C107, DICYT-C119, DICYT-C217, DICYT-C114 and DICYT-C186) and two Trinitarian type (CCN-51 and LR-35).

Thirty flowers were collected per clone (4 ramets out a total of 10), one day per week for 6 months. The collected flowers were transported in Petri dishes with 0.7 % agar solidified medium to avoid movement during transportation and were analyzed on the same day or preserved in the agar preparation at 0 °C temperature when done on a different day. To count pollen grains stigmas were cut off under magnifying glass and stained with fuchsine and methylene blue (modification of Alexander's stain) to obtain color contrast and facilitate counting. The modification of Alexander's method was made considering that staining membranes of pollen grains is a better indicator of viability than staining sporodermes.

Counting pollen grains was carried out under an optical microscope. The total number of pollen grains was determined by a total count of grains deposited on stigmas. Pollen grains that turned red were considered as viable and constricted, and unstained grains as nonviable (Osborn et al., 1988). In the present study pollen flow means the average amount of pollen grains found on stigmas per clone.

The data of maximum and minimum temperature, precipitation, relative humidity and wind speed were obtained from the climatic station of the experimental farm. The respective data was used each day in which flowers were collected.

The data were arranged in a factorial way and distributed in a completely random design with four repetitions (30 flowers per repetition), considering the different months and clones of evaluation as factors. The data referring to pollen flow were analyzed under the statistical program SAS (Statistical Analysis System), by applying the Tukey test. The correlation of pollen flow with the climatic variables was analyzed under a regression model and a scatter plot of points, using the Statistic 7.0 program.

RESULTS AND DISCUSSION

The flow of viable and nonviable pollen grains per flower was significantly different ($P \leq 0.000$) among clones, months and their interactions (Table 1).

The clone that obtained the highest average of total pollen grains per flower was LR35 with 20.89, and the one with the lowest average was DICYT-C119 with 9.96 pollen grains per flower (Table 2). The variation in pollen production between genotypes of the same species has already been documented (García et al., 2017).

The general average of 15.79 pollen grains per flower obtained from the seven clones is low if we consider that the plant produces a mean of 48 ovules per flower (generating the same number of seeds per pod), and indicates that only a small percentage of the produced flowers received enough pollen to form a fruit. The low pollen flow registered in the seven clones of *T. cacao* would be one of the main limitations in the production of fruits in cacao orchards.

The relevant literature does not mention pollen flow studies for *T. cacao*, but numerous studies in other species have shown that insufficient pollen reception limits seed production in plant populations (Osborn et al., 1988; Ashman et al., 2004; Knight et al., 2005).

The viable and nonviable pollen grains were significantly different between clones, with averages that ranged between 7.89 and 16.87 viable pollen grains for clones DICYT-C119 and CCN51, and averages between 1.96 and 3.29 nonviable pollen grains for clones DICYT-C114 and DICYT-C107 respectively. February was the month in which more pollen grains per flower were recorded on stigmas with 24.90 pollen grains (average obtained from weekly data). The month with the lowest value was December with 9.06 pollen grains per flower (Table 2).

It is of interest to note that February was the time of the year showing the best characteristics regarding the natural arrival of pollen in all the studied clones, both for the variable of total pollen grains and viable pollen grains (Table 2). The month that presented the lowest value was October followed by December for the total of deposited pollen grains. For the evaluated six months, the variable nonviable pollen grains ranged between 1 and 4 grains.

Table 1. Square means of pollen flow by flower in seven genotypes of *T. cacao* during the six months in the experimental farm La Represa, Province of Los Ríos, Ecuador. Years 2015-2016

Source of variation	df	Total pollen grains	Viable pollen grains	Nonviable pollen grains
Clone	6	67,345	11,224	33.52
Month	5	137,339	27,468	82.03
Clone x month	30	116,501	3,883	11.59
Experimental error	4,998	1,673,463	335	
Total	5,039			
P clone		<.000	<.000	<.000
P month		<.000	<.000	<.000
P clone x month		<.000	<.000	<.000

df: degrees of freedom; *P*: significance level

Table 2. Pollen flow by flower in seven genotypes of *T. cacao* during the six months in the experimental farm La Represa, Province of Los Ríos, Ecuador. Years 2015-2016

Clones	Total pollen grains	Viable pollen grains	Nonviable pollen grains	n
CCN51	19.40 ab	16.87 ab	2.53 ac	720
LR-35	20.89 a	18.25 a	2.64 b	720
107	12.27 cd	8.98 c	3.29 abd	720
114	13.88 c	11.92 e	1.96 ac	720
119	9.96 d	7.89 c	2.06 cd	720
186	16.39 ce	13.18 de	3.21 abcd	720
217	17.80 be	15.10 bd	2.70 d	720
Month				
September	17.56 a	14.87 a	2.70 a	840
October	10.60 b	9.50 b	1.10 b	840
November	14.80 c	12.64 a	2.16 a	840
December	9.06 bc	7.44 b	1.63 b	840
January	17.86 a	13.60 a	4.26 c	840
February	24.90 d	20.99 c	0.83 b	840

Different letters indicate statistic differences according to Tukey test ($P \leq 0.05$); n: number of observations

A low amount of nonviable pollen grains was registered in the stigmas of flowers corresponding to the evaluated clones. The clone that presented more pollen grains, both total and viable deposited on stigmatic surface during the evaluation period was LR35 where between 9.40 and 27.5 pollen grains were counted, with an average of 18.25 viable pollen grains. While clone DICYT-C119 had the lowest arrival of pollen (an average of 10 grains of which 80 % were viable) it can be noted that this clone has been categorized as non-productive, according to historical reports (Osborn et al., 1988).

Maximum temperature showed a moderate positive correlation with the amount of pollen grains per flower observed on stigmas (r = 0.46, *P* = 0.04). However, minimum temperature did not determine the amount of pollen grains per flower observed on stigmas, since no relationship was observed between these two variables (r = 0.13; *P*= 0.57). The incidence of pollinators seems to be at the time of the day with the highest air temperatures (Figure 2). The climatic data allowed knowing that there was a moderate positive correlation between maximum temperature and the natural arrival of pollen; there is also a positive correlation, although low, with other climate factors such as relative humidity, precipitation and wind speed. This low correlation could be attributed to a higher air humidity prevailing within the plantation above values recorded at the weather station in Quevedo.

On the other hand, it should be taken into account that the air humidity is important to keep the viability of the pollen grains. The highest recorded temperatures detected in January and February (rainy season) could be the weather factor associated to the greater number of pollens found on stigmas. Visual observations point toward a greater number of flowers during the rainy season that attract more pollinators increasing, in turn, the pollen deposition in flowers within short distances (Meyer et al., 2009).

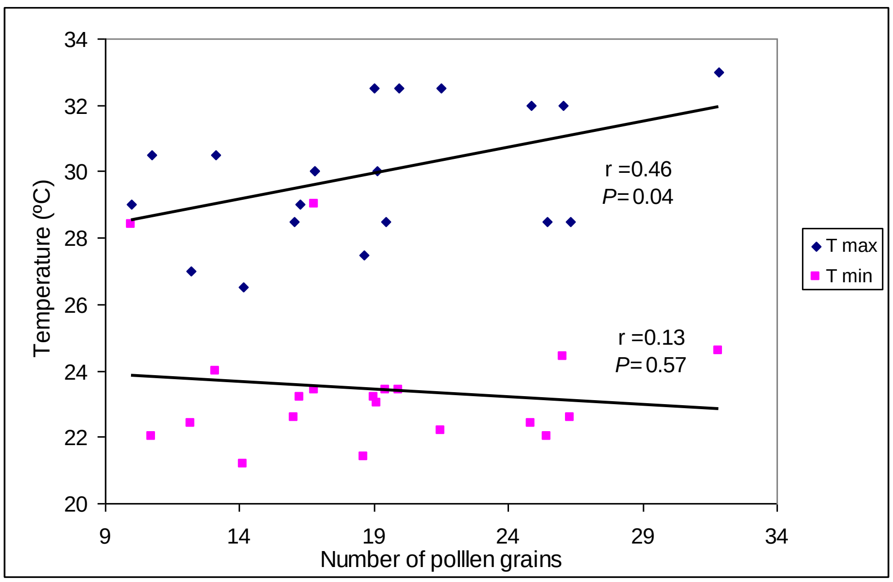

Figure 2. Correlation between temperature and pollen flow in seven genotypes of *T. cacao* in the experimental farm La Represa, Province of Los Ríos, Ecuador. Years 2015-2016 (120 flowers per month during six months); n=720 per clone

There was a significant (*P*=0.036) low positive correlation (r = 0.33) between the precipitation (rainfall) recorded in the months of study and total pollen grains (Figure 3); similarly, it existed a low positive correlation between the relative humidity and the number of pollen grains per flower (r = 0.27) but with lower significance level (*P*≤ 0.086). Climatic factors such humidity and light incidence influence pollination. Nevertheless; this process does not occur immediately, it should also be considered that rainfall affects the fall of pollen and therefore of flowers (De la Cruz and Soria, 1973). Therefore, for plant species pollinated by insects reproductive success and genetic exchange through pollen transfer depend on efficiency, abundance and behavior of floral visitors (Meyer et al., 2009). In this case, the biological cycle of pollinator and how the meteorological parameters affect it should be considered not only in plants but also in the other themes. According to Armijos et al. (2016) there is a moderate positive correlation between the presence of insects and relative humidity, although it is necessary to study the complete life cycle of pollinator and its relationship with climatic variables.

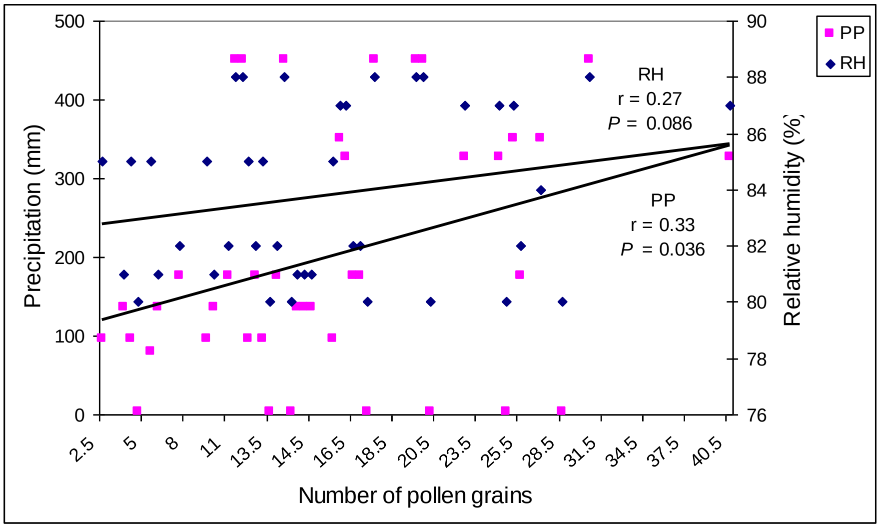

Figure 3. Correlation between pollen flow, precipitation (PP) and relative humidity (RH) in seven genotypes of *T. cacao* in the experimental farm La Represa, Province of Los Ríos, Ecuador. Years 2015-2016 (120 flowers per month during six months); n=720 per clone

Although *T. cacao* is an entomophile species, there was a positive low correlation (r = 0.29) with low significance level ($P \leq 0.061$) between wind speed and pollen amount that reaches to stigmas (Figure 4). Effective pollination depends on a large extent to the synchronization of dynamic populations of Diptera with flowering cycles of trees and the abundance of mosquitoes is related to flower quantity (Young, 2008). In this regard, Armijos (2016) recorded a very low population of pollinating insects belonging to the family of Drosophilidae and Cecidomyiidae in the same place of our study (only 3.29 % of these families in relation to the total number of insects found). This low presence of pollinators would explain the low pollen flow in the evaluated trees, considering that pollen grains in *T. cacao* are sticky, which makes it difficult for wind to drag it (Young, 2008).

In general, a low pollen reception was recorded in four clones during the study period. The highest values were found in LR35 in which 20 % of the flowers received 25 or more pollen grains, while in DICYT-C107 only 4.4 % of the flowers collected enough pollen grains to produce an ear (fruit), in the event that each grain germinates and fecundates an ovule (Table 3).

In the related species *T. speciosum*, 60 % of pollen grains found on receptive stigmas were viable (De Souza and Venturieri, 2010). These results are lower than those recorded in the present study where this variable ranged from 73 to 87 % of viable pollen in *T. cacao*. These data reveal that pollen quality was not a limitation for the production of the evaluated *T. cacao* clones, since a high viability was found in pollen grains found on stigmas in the studied clones. Although the amount of pollen is important its quality was not less. The quantity and quality of pollen transferred from anthers to stigmas varies substantially between plants, with consequences for fertility, mating system and hybrid vigor (Aizen and Harder, 2007).

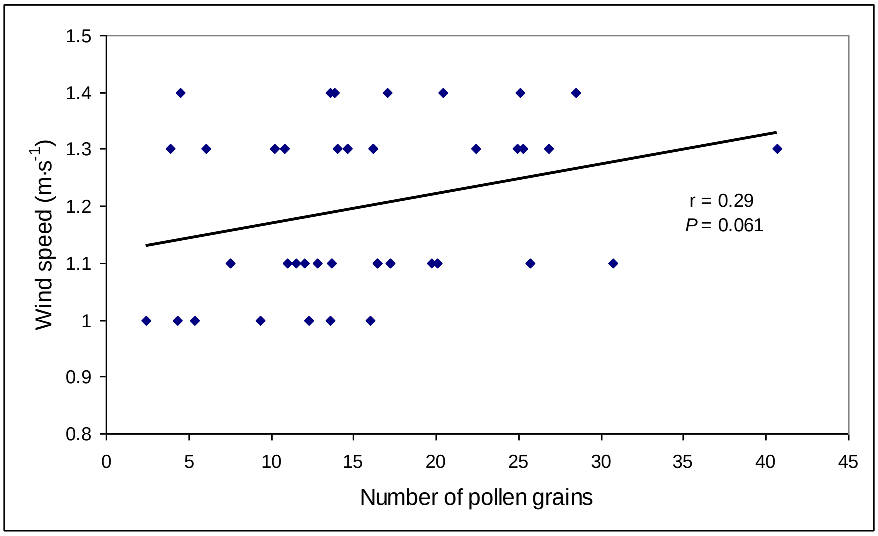

Figure 4. Correlation between pollen flow and wind speed in seven genotypes of *T. cacao* in the experimental farm La Represa, Province of Los Ríos, Ecuador. Years 2015-2016 (for six months, 120 flowers per month); n = 720 per clone

Table 3. Percentage of flowers with more than 25 pollen grains in the clones of *T. cacao* in the experimental farm La Represa, Province of Los Ríos, Ecuador

	DICYT-C107		DICYT-C119		DICYT-C217		DICYT-C114		DICYT-C186		CCN51		LR35	
Month	T	%	T	%	T	%	T	%	T	%	T	%	T	%
Sep.	1	0.8	1	0.8	29	24.1	19	15.8	29	24.1	36	30	1	0.8
Oct.	0	0	16	13.3	21	17.5	13	10.8	12	10	0	0	20	16.6
Nov.	7	5.8	0	0	13	10.8	24	20	3	2.5	1	0.8	35	29.1
Dec.	0	0	1	0.8	6	5	0	0	22	18.3	16	13.3	22	18.3
Jan.	1	0.8	8	6.7	11	9.16	0	0	9	7.5	16	13.3	26	21.7
Feb.	23	19	29	24.1	32	26.6	7	5.8	8	6.7	55	45.8	41	34.2
Prom		4.41		7.6		15.5		8.7		11.5		17.2		20.1

T = Total of flower >25 pollen grain

The arrival of pollen grains to stigma does not imply fertilization. the counting of pollen grains should not be extrapolated to the production of fruits nevertheless, we considered that there was the possibility that a flower could turn into fruit when at least it had received 25 grains of pollen (Somarriba et al., 2010) (notwithstanding that the authors have observed cobs in the field with few pollen grains, although their production would be unprofitable). in the present study only a low percentage of flowers in the evaluated clones receives the amount of 25 or more pollen grains, the national type clones received less pollen than those of the Trinitarian type, this could be due to the variable flower production (Meza, 2016) and pollen in the different genotypes.

CONCLUSIONS

Pollen flow per flower of *T. cacao* in the studied area is insufficient to promote a high percentage of tied fruits (effective pollination).

Pollen flow the crop is influenced moderately by the air maximum temperature, and with lesser extend by wind speed. The rainfall and relative humidity have some effects as well, which would justify the greater presence of pollen in the rainy season. The minimum temperature does not have effect on the amount of pollen grains on stigmas.

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