POLLINATION DEFICIT IN OPEN-FIELD TOMATO CROPS (*SOLANUM LYCOPERSICUM* L., SOLANACEAE) IN RIO DE JANEIRO STATE, SOUTHEAST BRAZIL

Mariana Scaramussa Deprá[1], Geovana Carla Girondi Delaqua[1], Leandro Freitas[2], Maria Cristina Gaglianone[1]*

[1]*Laboratório de Ciências Ambientais– LCA, Programa de Pós-Graduação em Ecologia e Recursos Naturais, Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF, Campos dos Goytacazes, Rio de Janeiro, Brasil*
[2] *Jardim Botânico – IP/JBRJ, Rio de Janeiro, Rio de Janeiro, Brasil*

Abstract— More than 70% of world's crops benefit from biotic pollination, and bees are their main pollinators. Despite the fact that some of these insects have been broadly studied, understanding the interactions between plant crops and their pollinators with a local scale approach is necessary when aiming to apply proper protective and management measures to pollinators and their respective crops. In this context, we analyzed the pollination status of open-field tomato crops (*Solanum lycopersicum* L.), regarding fruit-set, visitation rate and the quality of fruits. We recorded the formation of fruits through spontaneous self-pollination and open-pollination, and the occurrence of pollinators in 24 areas of open-field tomato crops. We performed experiments of apomixis, spontaneous self-pollination, manual cross pollination and supplemental cross pollination (simulating the pollinator behavior) in a greenhouse. The fruit quality was evaluated according to circumference, weight, volume and number of seeds. Higher production of fruits after open-pollination compared to spontaneous self-pollination indicates the importance of pollinators to increment productivity of *S. lycopersicum* in the study area. The circumference and the number of seeds from tomatoes of the greenhouse plantation did not differ between spontaneous self-pollination and the manual cross pollination. In the open-field crops the number of seeds was higher for fruits resulting from open-pollination. Our results indicate that the importance of bees is mainly related to the increase in fruit production, thus incrementing the productivity of tomato crops.

Keywords: bees, buzz pollination, environmental services, fruit-set, fruit quality

INTRODUCTION

The decline of pollinators and the losses associated to the pollination service have been recorded in several countries, and one of the greatest challenges to solve this problem is to search for measures that aim at the conservation of the biodiversity allied to crop production. More than 70% of world's crops benefit from biotic pollination, and bees are their main pollinators (Klein et al. 2007). In this sense, understanding the interactions between plant crops and their pollinating fauna under a local scale approach is necessary when aiming to apply proper protective and management actions to pollinators and their respective crops (Klein et al. 2003).

Most of the knowledge on the biodiversity of bees comes from studies in natural remnants of Brazilian ecosystems, like Atlantic Forest (Imperatriz-Fonseca et al. 2012). In crop areas, the biodiversity knowledge is still incipient, with studies restricted to a few cultures and areas (Yamamoto *et al.*, 2010). Some of these studies focused on plantations of west indian cherry (Freitas et al. 1999), cashew (Freitas & Paxton 1998; Freitas et al. 2002), coffee (De Marco & Coelho 2004), apple (Souza 2003) and passion fruit (Camillo 1998; Benevides et al. 2009; Yamamoto et al. 2012).

This scenario is even more concerning when one considers that forest fragmentation - with a large fraction caused by the expansion of agriculture - reduces the heterogeneity of habitats, and consequently, rapidly changes the composition and diversity of pollinators (Benton et al. 2003). Within a short time, natural complex ecosystems are transformed into agricultural areas and many of them are simplified to less heterogeneous systems (Tscharntke et al. 2005).

The relationship between biodiversity in natural ecosystems and agricultural areas is particularly important when the pollinating bees are considered. These insects demand food and nesting resources which most often are not provided in agroecosystems. The forest fragments are used as mating and nesting areas, and also provide several other resources for many bee species (Chacoff & Aizen 2006). These facts can explain results that demonstrate that the proximity to forest fragments may contribute to higher diversity of pollination agents in crop areas and increase the pollination efficiency of cultivated plants (Kevan &

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*Corresponding author; email: mcrisgag@uenf.br

Imperatriz-Fonseca 2002; Kremen et al. 2004; Chacoff & Aizen 2006; Klein et al. 2007; Benevides et al. 2009; Garibaldi et al. 2011). Besides that, not only the productivity but also the quality of fruits, in addition to resistance to environmental conditions and diseases, can be improved through cross pollination performed by bees (McGregor 1976).

When generally considered, limitations on the quantity and quality of fruits of crops may define problems regarding pollination deficit, which consists of the difference between the optimal level of pollination and the real pollination, and is mainly a result of insufficient visitation of pollinators (Wilcock & Neiland 2002; Vaissière et al. 2011). In this context, we aim to evaluate the occurrence of pollination deficit in open-field tomato crop areas in Southeast Brazil.

MATERIALS AND METHODS

Studied system

The tomato (*S. lycopersicum* L., Solanaceae) is an annual herbaceous species, grown in several regions of Brazil. The Rio de Janeiro state is responsible for about 200 thousand tons/year, corresponding to 5% Brazil's tomato production (IBGE 2010). Some different cultivars are planted in the study area, but mostly two cultivars are used: Ivanhoé Agrocinco® and Dominador Agristar®.

The tomato flowers are hermaphrodite, small (1-2cm of diameter), with yellow corolla and anthers, presenting five free stamens, with anthers forming a cone around the stigma (Minami & Haag 1989). Similar to most *Solanum* species, the anthers are filled with small and dry pollen grains, which are released through apical pores (Buchmann 1983). The morphology of the anthers and pollen grains allows for the collection of pollen by bees performing buzz pollination (Buchmann & Hurley 1978), by vibrating the flowers.

Study Area

We studied areas of open-field tomato crops (n = 24) with 0.5 to 4.5 ha in the municipality of São José de Ubá, Rio de Janeiro State, Southeast Brazil (42°04' to 41°55'W; 21°21' to 21°30'S), Fig. 1. The region presents a warm tropical climate, with dry winter (April to September) and rainy summer (October to March), with mean annual temperature around 23°C. The average annual precipitation is 1,200mm (Gonçalves et al. 2006). The original vegetation of the region was mainly lowland semi-deciduous forest, belonging to the Atlantic Forest biome (Dan et al. 2010). Nowadays only 4% of the original vegetation remains in this region (less than 1,000 ha), distributed in small fragments, with less than 10ha each, most of them on the top of hills (Fundação SOS Mata Atlântica 2008).

The cultivation in the area follows the system with stalks and traditional techniques (Moura 2005), under similar cycle of pesticide and irrigation: the agrochemicals are applied in the afternoon three times a week and irrigation is performed every two days (M. S. Deprá pers. obs.). All samplings and experiments were conducted in the middle of the crop areas.

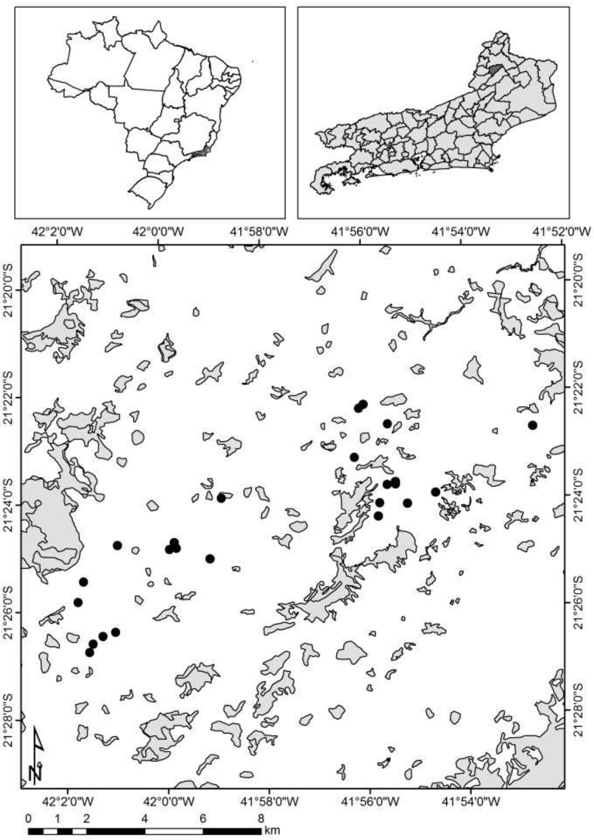

FIGURE 1. Location of the 24 studied crop areas (black circles) in northwest Rio de Janeiro state (map above right) in Brazil (left). Grey areas indicate forest fragments in the region.

We conducted part of the experiments of manual pollination in a greenhouse, with no control of climate conditions, in the city of Campos dos Goytacazes, located about 80Km from the open cultivated areas, with similar climatic conditions (RadamBrasil 1983).

Composition and Frequency of Floral Visitors

To verify the frequency of visits to tomato flowers, we performed two different methodologies in 2010 and 2012. In July-August 2010, we searched for visiting bees by walking through the lines of the cultivated areas (n=16), from 8 am to 2 pm, during three sampling times of 15 minutes in each hour, totalling 9 sampling hours per area by two collectors at the same day. In July-August 2012 flower visiting insects were monitored in 120 plants by four collectors from 10am to 1pm, during six sampling times of 15 minutes in two days at the same area, totalling 6 sampling hours per area.

We evaluated the rate of visitation through the frequency of pollinators and the number of flowers in each tomato crop area studied, on the same days when the pollination experiments were conducted. Visitors were considered legitimate pollinators when they collected pollen by buzzing the flowers, contacting the reproductive parts of the plants; we considered non-buzzing visitors as robbers. The number of open flowers in each area was calculated based on the counting of all open flowers from 30 individuals in each area, then calculating the average number of flowers/plant. After that, the rate of visitation was calculated as: number of pollinators sampled during the day/number of observed flowers/time of observation.

In order to compare the composition and importance of the different pollinators of tomato between the two study years, we calculated the relative frequency of visitors summing all studied areas in each year. Vouchers of the visiting bees were deposited in the Zoological Collection of Laboratory of Environmental Sciences at the 'Universidade Estadual do Norte Fluminense Darcy Ribeiro', in Campos dos Goytacazes, RJ, Brazil.

Pollination Experiments

We performed the experiments of open-pollination and spontaneous self-pollination in 24 crop areas, between July and August in 2010 and 2012. In order to evaluate the natural pollination rates, we marked 1,850 flowers and exposed them to pollinators (open-pollination). We tested spontaneous self-pollination by marking 1,858 pre-anthesis buds bagged with soft tissue, preventing the pollinators to contact the flowers. We evaluated fruit-set or fall of the floral receptacle after 10 days of each treatment.

In a greenhouse, we planted 40 tomato plants of the same cultivars evaluated in the open field (Ivanhoé Agrocinco® and Dominador Agristar®). Flowers going through pre-anthesis were isolated and used in the following pollination experiments: 1) Apomixis (APO): flower buds were emasculated and bagged in order to prevent the arrival of self-pollen to the stigma (*n*=10 flowers); 2) Spontaneous self-pollination (SSP): flowers were kept bagged until a fruit was set or the receptacle fell (*n*=69 flowers); 3) Manual cross-pollination between cultivars (CBC): a load of pollen from three different individuals of a cultivar of tomato was placed on the stigma of a flower of the other cultivar, which had been previously emasculated (*n*=122 flowers); 4) Manual cross-pollination in the same cultivar (CSC): a load of pollen from three different individuals of a cultivar of tomato was placed on the stigma of a flower of the same cultivar, which had been previously emasculated (n = 123 flowers); 5) Supplemental manual cross-pollination (SCP): the same treatment of CBC + CSC, without previous emasculation (n = 156 flowers).

We evaluated the success of the treatments according to the percentage of fruit-set (fruits/flowers). For the analysis of quality of fruits from the manual pollination experiments in greenhouse, we measured the widest circumference (cm) and the number of seeds per fruit (Tab. 1), 30 days after the treatments.

In order to evaluate the quality of fruits (Tab. 1) from the experiments performed in the field, we measured the weight (g), volume (ml) and widest circumference (cm) and we counted the number of seeds in fruits collected 70 days after the treatments, which corresponds to the average time for the farmers to collect the fruits.

TABLE 1. Number of fruits of tomato measured for each parameter of quality after pollination treatments, performed in open-field crops (São José de Ubá) and in a greenhouse (Campos dos Goytacazes). SSP = spontaneous self-pollination, SCP = supplemental manual cross pollination, CSC = manual cross pollination in the same cultivar, CBC = manual cross pollination between cultivars

Open-field crop	
Treatment	Sampled size Widest circumference, weight, volume, number of seeds
Open-pollination	64
Spontaneous self-pollination	54

Greenhouse		
	Sampled size	
Treatment	Widest circumference	Number of seeds
SSP	14	33
SCP	74	99
CSC	43	33
CBC	41	52

Data Analyses

We analysed the percentages of fruit-set through the Partitioning Chi-square test (X^2), with the software BioEstat 5.0 (Ayres et al. 2007). We used Spearman correlation to evaluate the relationship between fruit-set under natural pollination and rate of visitation of pollinators in each area.

We compared the fruit measures (weight, widest circumference, volume and number of seeds) from the experiments of pollination performed in the field (open-pollination vs. spontaneous self-pollination) using the non-parametric Wilcoxon-Mann-Whitney test (U Test). We used Kruskal-Wallis and the after test of Dunn to compare the weight and the widest circumference of fruits from the pollination tests conducted at the greenhouse. These tests were performed in the software Statistica 8.0 (StatSoft 2007).

RESULTS

Composition, frequency and visitation rate of pollinators

Bees were the only visitors of the tomato flowers and except for *Apis mellifera* and *Trigona spinipes*, all other bee visitors were considered pollinators because they buzz the flowers (Tab. 2). The species composition was similar between the two studied years and species of *Exomalopsis* were the most frequent pollinators in both years.

Fruit-set and quality of fruits

The percentage of fruit-set in the field resulted from the open-pollination was higher than that resulted from the self-pollination in the two years of study (Tab. 3). In 2010 the percentage of fruit-set from open-pollination presented a

TABLE 2. Visitors of *Solanum lycopersicum*, their behaviour and relative frequency in open field cultivated areas in Rio de Janeiro state, Brazil, evaluated in years 2010 (n=16 areas) and 2012 (n=8). Po = Pollinator; R = Robber.

	2010	2012	Behaviour
APIDAE			
Apis mellifera L.	0.247	0.063	R
Centris spp.	0.041	0.011	Po
Bombus morio Sw.	0.006	0.023	Po
Euglossa spp.	0.006	0.097	Po
Eulaema nigrita Lep.	0.009	0.011	Po
Exomalopsis spp.	0.229	0.487	Po
Melipona quadrifasciata Lep.	0.002	0.000	Po
Trigona spinipes Fab.	0.227	0.011	R
Xylocopa spp.	0.089	0.106	Po
HALICTIDAE			
Augochloropsis spp.	0.109	0.184	Po
Pseudaugochlora spp.	0.031	0.000	Po
ANDRENIDAE			
Oxaea flavescens Klug	0.004	0.008	Po

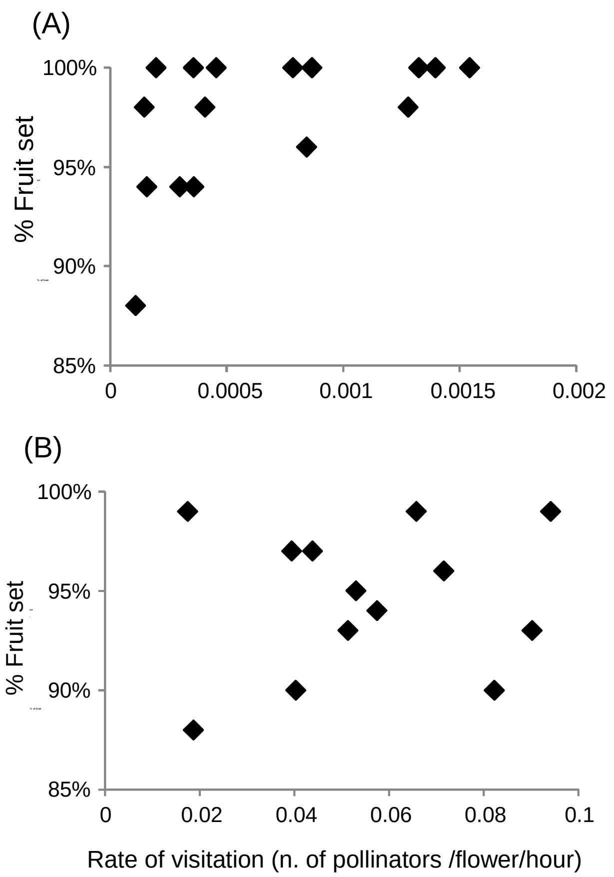

FIGURE 2. Relationship between fruit-set from open-pollination and rate of visitation (number of pollinators/flower/hour) in open-field tomato crops, São José de Ubá in 2010 (A) and 2012 (B).

positive correlation with the visitation rate of pollinators (rs= 0.57; *P*= 0.02; n= 16), which was not observed in 2012 (rs= 0.069; *P*= 0.82; n= 13) (Fig. 2). No fruit was set through apomixis in the greenhouse. The fruit-set after supplemental manual cross-pollinations (84%) was higher than after spontaneous self-pollination (55%) and in the other two experiments of manual cross-pollination (53% and 55%), which did not differ among them (Tab. 4).

In the field, the average number of seeds per fruit after open-pollination was higher than the ones observed for fruits from spontaneous self-pollination (*U*= 1291.5; Z= 2.74; *P*= 0.006), and no significant differences among treatments were detected for the other variables (Fig. 3).

In the greenhouse, both the average widest circumference of the fruits and the average number of seeds per fruit from the spontaneous self-pollination and supplemental manual cross-pollination treatments were higher than the ones obtained for both experiments of manual cross-pollination (CBC and CSC) (Fig. 4A and 4B).

DISCUSSION

In spite of the observed high percentages of fruit-set from bagged flowers, the greater production of tomato fruit after open-pollination (natural pollination) when compared to the spontaneous-self-pollination in open-field crops indicates the importance of pollinators to increase the productivity of *S. lycopersicum* in the study area. In addition, in one of the study years (2010), higher percentages of fruit-set were observed in plantations that presented higher pollinator visitation rate. The increase in fruit-set of tomato through bee pollination was also identified in other regions, like California (EUA) (Greenleaf & Kremen 2006) and Mexico (Macias-Macias et al. 2009), corroborating the importance of bees to pollinate that system. This idea is coherent with a lower fruit-set of tomato (50%) observed in an urban area in southwest Brazil, associated to the low number of floral visitors (Bispo dos Santos *et al.* 2009). In addition, the crop areas sampled in São José de Ubá that presented lower frequency of pollinators returned less than 90% fruit-set after open-pollination, similar to the values found after spontaneous self-pollination. These results suggest that there might be a critical value for bee abundance (reflecting on the frequency of visitations to flowers) for the pollination performed by these insects to be able to increment the fruit-set rate in tomato plants. The loss of pollination service has been observed for other crops in Brazil, for instance apple and melon. In those cases, the management of *Apis mellifera* colonies has been used in order to increase the rate of pollination (Freitas & Imperatriz-Fonseca 2005). The results we presented indicated that tomato, despite its self-pollination, may have its productivity reduced as a consequence of pollinator deficit.

In the greenhouse, the experiments showed absence of apomixis in both tomato varieties (Ivanhoé Agrocinco® and Dominador Agristar®). The comparison between the percentage of fruit-set in the supplemental manual cross-pollination (simulating the behaviour of pollinators) and in

TABLE 3. Comparison between the percentage of fruit-set from open-pollination and spontaneous self-pollination, conducted in open-field tomato crops in São José de Ubá, in 2010 and 2012. Values in parentheses indicate number of fruits/tested flowers. * indicates significant difference between tests in each area (P<0.05)

Years	Percentage of fruit-set		Chi-square (X^2)		
	Open-pollination	Spontaneous self-pollination	X^2	Degrees of freedom	P-value
2010	97,5% (780/800)	90,2% (722/800)	36.56	1	< 0.0001*
2012	95,3% (1001/1050)	87,9% (930/1058)	37.84	1	< 0.0001*

TABLE 4. Comparison between the percentages of fruit-set resulting from manual pollination experiments in a greenhouse. APO= apomixis; SSP= spontaneous self-pollination; SCP= supplemental manual cross pollination; CSC= manual cross pollination in the same cultivar; CBC= manual cross pollination between cultivars. Values between parentheses indicate number of fruit produced/tested flowers. Different letters indicate statistical difference according to Partitioning Chi-square (X^2 = 37.17, df = 3, P < 0.0001), which was detected after considering the SCP treatment in comparison to the rest of the table

Treatment	Percentages of fruit-set
APO	0 (0/10)
SSP	55% (36/65)^A
SCP	84% (123/147)^B
CSC	53%(62/117)^A
CBC	55% (63/115)^A

Partition	X^2	Degrees of freedom	P-value
CSC:CBC	0.0807	1	0.7763
(CSC+CBC):SSP	0.0499	1	0.8232
(CSC+CBC+SSP):SCP	37.0359	1	<0.0001

the spontaneous self-pollination corroborated the data on open- pollination and spontaneous self-pollination observed in the open-field tomato crops, demonstrating that pollinating bees are able to increase fruit-set. Other studies verified increment on the fruit-set of tomato plants in greenhouses, when the flowers were pollinated by bees (Al-Attal et al. 2003; Palma et al. 2008; Bispo dos Santos et al. 2009).

The fruit-set rates observed after spontaneous self-pollination were higher in the open-field tomato crops than in the greenhouse. This result is likely to be influenced by the wind in the field, which enhances the efficiency of spontaneous self-pollination, due to its capability of swinging the flowers promoting the release of pollen grains from the anthers (McGregor 1976). However, the deviation of results caused by methodological procedures cannot be discarded; different from the observations in the field, the plants were daily observed in the greenhouse, when was possible to observe that part of fruits fell after 10 days of observation. That observation indicates that the percentages of self-pollination in the experiments held in the greenhouse could be higher than the ones recorded.

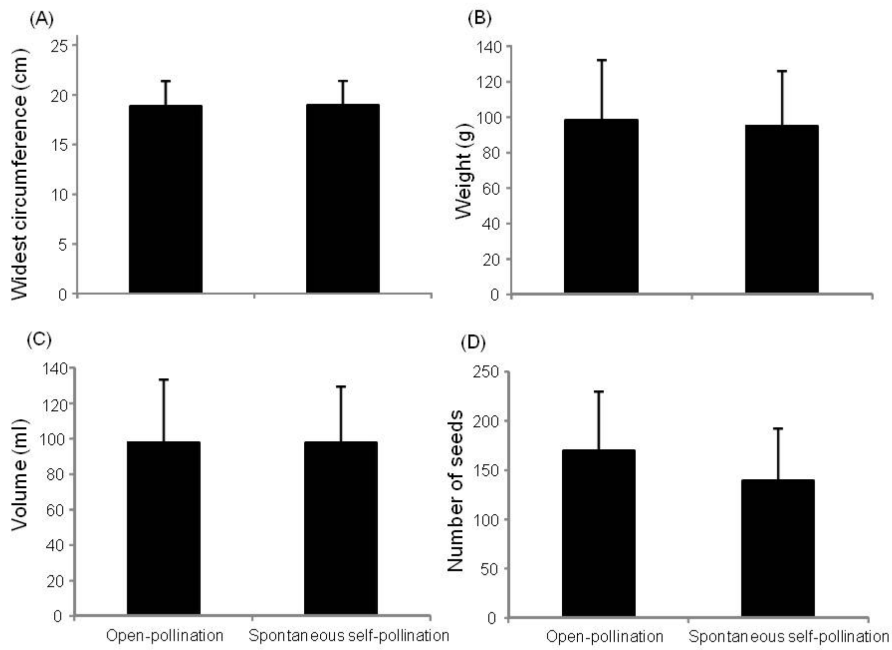

Figure 3. (A) Widest circumference (cm), (B) weight (g), (C) volume (ml) and (D) number of seeds (mean ± SD) of tomatoes from experiments of open-pollination and spontaneous self-pollination in open-field crops in 2012.

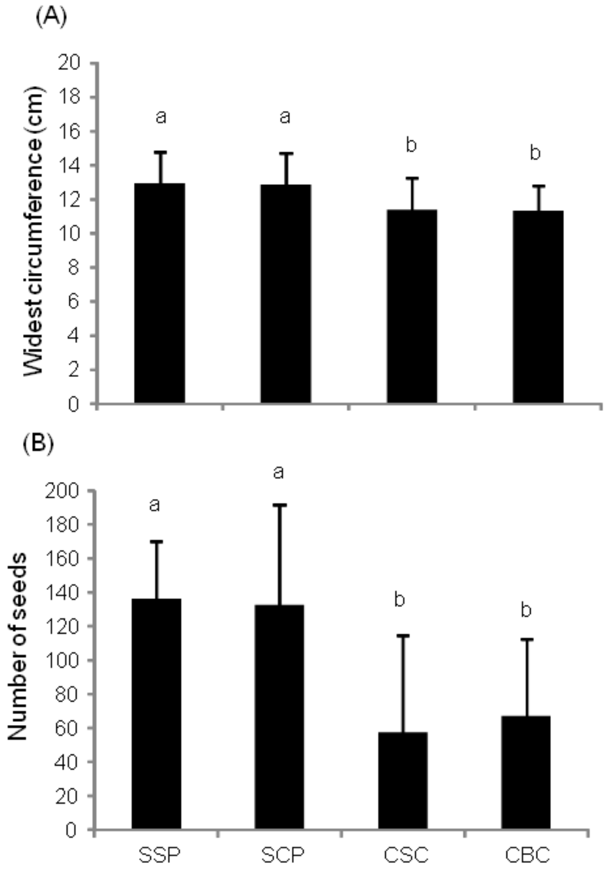

FIGURE 4. (A) Widest circumference (cm) and (B) number of seeds of tomato fruits (mean ± SD) from the pollination tests performed in the greenhouse. Spontaneous self-pollination (SSP), supplemental manual cross pollination (SCP), manual cross pollination in the same cultivar (CSC) and manual cross pollination between cultivars (CBC). Different letters indicate different means according to the Kruskal-Wallis Test ($P < 0.05$).

The two parameters used to evaluate the quality of fruits (the widest circumference and the number of seeds), from the greenhouse, did not differ between fruits resulted from spontaneous self-pollination and supplemental manual cross-pollination, which means that no significant difference in quality was observed for fruits originated from self-pollination and from the action simulating the pollinators. The absence of difference had also been recorded for the same parameters regarding fruits from self-pollination and from manual vibration and vibration performed by bees (Del Sarto *et al.* 2005). Other studies reported wider circumference and greater number of seeds for fruits originated from bee pollination (Dogterom et al. 1998; Aldana et al. 2007; Bispo dos Santos et al. 2009; Hikawa & Miyanaga 2009; Macias-Macias et al. 2009). Finally, the fact that the measures were taken before the whole development of fruits could have biased our results.

In the open-field tomato crops, the number of seeds was higher for fruits originated from open-pollination. A similar result was also reported by Macias-Macias et al. (2009). The number of seeds is directly related to the number of pollen grains that arrive at the stigma, and consequently to fertilization of ovules, which increases with the aid of cross pollination performed by bees (Morandin et al. 2001; Kinet & Peet 2002). In a different way, parameters like circumference, weight and volume can reflect the ability to allocate resources for fruit development by plants. Therefore, the absence of significant variation among these parameters in our results may result from other factors like soil fertility and temperature (Minami & Haag 1989; Fandi et al. 2010).

According to the results here presented, the importance of bees is mostly related to the increase in fruit-set of tomato and consequently on the productivity of this crop. The majority of studies have demonstrated the importance of *Bombus* species on the pollination of *S. lycopersicum* (Dogterom et al. 1998; Al-Attal et al. 2003; Aldana et al. 2007; Palma et al. 2008). However, other native species are efficient pollinators of tomato flowers, such as bees of the genus *Exomalopsis*, *Xylocopa* and the tribe Augochlorini, which are the main pollinators of open-field tomato crops in São José de Ubá.

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