The Importance of Bees for Eggplant Cultivations (Hymenoptera: Apidae, Andrenidae, Halictidae)

by

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ABSTRACT

The increasing demand for food and the "Pollination crisis" have emphasized the importance of better understanding the potential of different wild bee species for pollinating crops. The aim of this study was to investigate how dependent *Solanum melongena* L. is on bees for fruit production and if it is possible to observe any insufficiency of pollination in four (two organic and two conventional) eggplant cultivations. Bee samplings were performed during the eggplant's peak flowering. Three pollination tests (T1= without insect visitation; T2= free insect visitation; T3= Pollen complementation) were carried out in order to evaluate the importance of bees for fruit setting in *S. melongena* L. Most of the bee species collected on eggplant flowers were buzz-pollinators – *Bombus*, *Xylocopa*, *Exomalopsis*, *Centris*, *Oxaea* and many species of Halictidae, and can promote the eggplant pollination. *Trigona* sp. and *Apis mellifera* were also collected on flowers, but they can't vibrate their anthers, although *Apis* presented a flying adaptation while visiting the flowers and eventually can pollinate the flowers. Most of the unvisited flowers (T1) failed to form fruits and when it happened, those ones were much lighter and smaller than those formed from flowers of T2 and T3; demonstrating the importance of bees for eggplant pollination. No statistical differences were found between the weight of eggplants in T2 vs. T3 within each area, however, the weight of fruits from T2 tests varied and differed significantly between the four studied areas. Our results indicated no pollen insufficiency in the studied areas, although the use of pesticides may disrupt crop-pollinator interactions, which may cause pollination insufficiency. Furthermore, land

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management seems to be a factor that determines efficiency of pollination in agricultural landscapes and ensures pollination services in cropped areas.

INTRODUCTION

In Tropical Regions, about 25% of crops are considered dependent on bees for pollination and, consequently, for fruit setting and economically viable seeds (Heard 1999; Richards 2001). In a global scale, it is suggested that one third of the food consumed by humans depends directly or indirectly on pollinators (Williams 1995; Klein *et al.* 2007; Ollerton *et al.* 2011).

In the past decades, many species of pollinators have disappeared from agricultural areas (Allen-Wardell *et al.* 1998; Corbet 1991; Kearns *et al.* 1998; Kevan & Phillips 2001; Steffen-Dewenter *et al.* 2005; Williams 1994), in particular the population of honeybees, which has declined all around the world. In North America, at the end of the 90s, this decline was so abrupt that the abundance of pollinators was significantly reduced when compared with any period over the past fifty years (Allen-Wardell *et al.* 1998). In fact, in the early 90s, the IUCN/SSC (World Conservation Union/ Species Survival Commission - committee responsible for formalizing the extinction of species) estimated for the next decades, a worldwide loss of more than 20,000 species of plants; this loss was largely attributed to the decline of co-dependent pollinators (Heywood 1993).

The increasing demand for food and the "Pollination crisis", which could affect food production all around the world, have emphasized the importance of better understanding the potential of different wild bee species on pollinating crops (Hein 2009).

The knowledge about pollinator species related to a crop is the first step for the land management in order to preserve the services of pollination. Many species of cultivated plants have particular traits, which demand different pollinators with different size and foraging behavior for a proper pollination, (e.g. eggplants) (*Solanum melongena* L.). Like most plant species of genus *Solanum*, eggplants have poricidal anthers, which require specialized behavioral and morphological adaptations by potential pollinators (Avanzi & Campos 1997). In this species, the length of stamens is about one centimeter, with very short filaments and long bright yellow anthers; each one presenting two apical pores. Effective eggplant pollinators can acquire pollen by vibrating the anthers.

S. melongena L. is native to the tropical regions of Asia and has been cultivated for centuries by Arabs and Chinese. Its consumption is increasing mainly in Europe and the United States (Vieira *et al.* 1996), where it represents an important crop.

In Brazil, the consumption of this vegetable has increased due to the medicinal properties attributed to the eggplant (Gonçalves *et al.* 2006; Guerra *et al.* 2007) as well as its nutritional content. According to the data of CEASA – Campinas (2007), the production of eggplants in the State of São Paulo (Brazil) reaches 47,549 tons/year and is responsible for generating more than one thousand jobs.

Given the importance of eggplant cultivation, both in terms of nutrition and economic value, and considering the increasing need for alternatives that guarantee the sustainability of agricultural systems, more studies are required in order to obtain more information on the composition of pollination guilds, pollination efficiency, as well as the pollinators' responses to land management. In this study we aimed to answer two main questions: (1) how dependent is *S. melongena* on bees for fruit production? (2) Is it possible to observe any insufficiency of pollination in eggplant plantations?

MATERIAL AND METHODS

Study areas

The studies on the importance of bees to fruit set and the effects of crop management to fruit quality were conducted in two organic and two conventional commercial plantation of *Solanum melongena* L., Napoli cultivar, located in São Paulo State, Brazil (Table 1). The Köppen climate classification of the region is "Cwa" (humid subtropical climate with dry and mild winters from April to September and warm and wet summers, from October to March) (Teixeira 2004).

Sampling of bees

Samplings were performed in sunny days, between 7:00 and 18:00 h, during the peak of flowering period, in a total of 48 hours in each area. The collector walked along a line through the plantation and observed groups of six flower-

Table 1. Crop management, localization, number of specimens cultivated and period of study in the four studied areas.

Area: Localization (Crop management)	Coordinates	Studied Period	Number of plants on cropped area
I: Corumbataí (Conventional)	22° 13'15.33" S 47° 37'18.95" W	February to April, 2010	2000
II: Corumbataí (Conventional)	22° 14'29.34" S 47° 36'40.04" W	September to November, 2010	1000
III: Limeira (Organic)	22° 34'57.00" S 47° 27'36.95" W	May to July, 2010	600
IV: Ajapí (Organic)	22° 18'39.46' S 47° 32'29.55" W	September to November, 2011	600

ing plants at a time, for about 5 to 10 minutes. Visiting bees were observed and their behavior in flowers was recorded. They were then collected with an entomological net, killed in ethyl acetate, dry mounted and identified until genus. We used Silveira *et al.* (2002) key to identify the bees.

Pollination Tests

In order to evaluate the importance of bees for fruit formation in *S. melongena* and to identify insufficiency of pollination in the crops, a series of tests (T1, T2 and T3) were carried out. In order to standardize the experimental design, the tests were conducted in plots positioned in the center of each cropped area and delimited with six hundred specimens of eggplants, thus using the smaller plantation as reference (Vaissière *et al.* 2009).

All tests were realized during the peak of eggplant's flowering. For each test, 50 flower buds in pre-anthesis stage were used. Only long-styled flowers were included. In T1 (without insect visitation), buds were bagged one day before anthesis and flowers remained covered for one week to avoid visitation by insects during the fertile period. Flowers were then uncovered, marked on the pedicel and the fruit development was observed for one month after when they were harvested. In T2 (free insect visitation) flower buds were marked in the pedicel and left uncovered, free to insect visitation; fruit formation was observed for a month until ripening when the fruits were then harvested. In T3 (pollen complementation), the treatment was the same as in T2, except that extra pollen were deposited on the stigma of each flower around 48 hours post-anthesis, with the aid of a brush.

All the fruits formed in these tests were harvested, weighed and had the largest diameter and length measured.

Data analysis

Since there is a positive correlation between the number of seeds and the weight in eggplants (Gemmill-Herren & Ochieng 2008), the weight of the fruits was used to indicate pollination efficiency.

Data were log transformed to minimize the effects of variability. In order to verify if there was statistical difference among the mean weight of eggplants in the experiments, a two-way ANOVA and *a posteriori* Tukey HSD t-test were performed, in which the factors considered were the different treatments (free insect visitation [T2] vs. pollen complementation [T3]) and the different areas. This analysis allowed two different approaches: (1) to compare the mean weight of eggplants between the two treatments in each area separately and consequently to test the hypothesis of pollination insufficiency; (2) to compare the mean weight of T2 among areas in order to verify the effect of a specific land management on the weight of the eggplants and, consequently, on the pollination efficiency.

RESULTS

Sampling of bees

The species of bees collected in flowers of eggplants are presented in Table 2. Most of the visiting bees collected on eggplant flowers are species that can perform buzz pollination by vibrating the tubular anthers and releasing pollen through apical pores, such as those of *Bombus*, *Xylocopa*, *Exomalopsis*, *Centris*, *Oxaea* and many species of Halictidae. *Apis mellifera* and *Trigona* sp. are not able to vibrate their anthers - they collect pollen fallen on petals. *Trigona* sp. can also chew the peak of the anthers and *Apis mellifera*, besides collecting the pollen fallen in flowers, presents a flying adaptation in which they release the pollen by grasping the tip of the anther's cone and flying up and down shaking the flower.

Pollination Tests

Table 3 presents the percentage of fruit formed in the different treatments (T1, T2 and T3) and the mean weight, diameter and length of the fruits harvested in four study areas, while Table 4 and Fig. 2 present comparisons

Table 2. Number of bee specimens collected on eggplants (*S. melongena* L.) flowers and their pollen gathering behavior. - Absence of bees.

Bees collected	Pollen gathering behavior	Number of individuals			
		Area I	Area II	Area III	Area IV
ANDRENIDAE					
Oxaea sp.	Vibrating	2	1	1	4
APIDAE					
Apis mellifera	Theft	7	1	1	1
Bombus	Vibrating	24	-	1	1
Centris	Vibrating	1	-	-	1
Centris sp.1	Vibrating	3	-	-	-
Epicharis	Vibrating	1	-	-	-
Euglosisni	Vibrating	1	-	1	1
Exomalopsis	Vibrating	2	10	23	4
Thygater	Vibrating	-	-	5	-
Trigona	Theft	6	10	10	12
Xylocopa	Vibrating	4	15	1	3
HALICTIDAE	Vibrating	2	18	6	9

Fig.1. Top: Fruit produced from T2 test (free insect visitation); Bottom: fruits produced from bagged flowers (T1).

Table 3: Percentage of formed fruits and mean weight, diameter and length of fruits formed in different pollination tests and in different studied areas. T1: without insect visitation test; T2 = free insect visitation test; T3 = pollen complementation test; % FF= percentage of fruit set; W = weight (g) ; D = diameter and L = length (cm), -= none or only one fruit formed (no mean value).

Area	T1				T2				T3			
	% FF	W (g)	D (cm)	L (cm)	% FF	W (g)	D (cm)	L (cm)	% FF	W (g)	D (cm)	L (cm)
I	8	162.5	6.63	17.13	84	386.5	8.46	23.68	100	368.44	8.13	23.23
II	2	-	-	-	98	242.41	7.07	22.57	94	218.51	6.7	20.93
III	14	149.29	5.81	17.07	96	302.29	7.30	22.80	86	301.74	7.15	23.11
IV	0	-	-	-	82	492.14	8.02	25.26	72	535.39	8.53	26.46

Table 4. Comparisons of the mean weight of fruit set in different tests (T2 and T3) within each study area and comparisons of those values for fruit set in (T2) among those areas (two-way Anova and *a posteriori* test Tukey HSD t-test; T1 = without insect visitation test; T2 = free insect visitation test T3 = pollen complementation test).

Within each area			
	T1 mean±sd(n)	T2 mean±sd(n)	Tukey HSD p<0.05
I	386.50±85.14(40)	368.44±75.00(48)	ns
II	242.40±59.33(49)	218.51±67.42(47)	ns
III	302.29±119.13(48)	303.57±105.09(42)	ns
IV	466.92±108.47(26)	535.38±151.23(26)	ns
Among areas (T2)			
I vs. II			*
I vs. III			*
I vs. IV			*
II vs. III			*
II vs. IV			*
III vs. IV			*

Ns – no statistical significance *Tukey HSD p<0.05

between the mean weight of formed fruits in the different tests (T2 vs. T3) within each study area and the comparisons between those values for fruits formed in the free insect visitation test (T2) in different studied areas.

Most of the bagged flowers (T1) failed to form fruits and when it happened, those fruits were much lighter and smaller than those formed from flowers left free for bee visitation or with pollen complementation (T2 and T3, respectively, Table 3, Fig. 1). The percentage of fruit set in T2 (free insect visitation) and T3 (pollen complementation) were quite expressive in all properties (Table 3). However, when comparing T2 and T3 at area I, it is possible to observe an increase in the percentage of fruits formed from pollen complementation (T3).

The results of statistical analyses are shown in Table 4 and Fig. 2. The two-way ANOVA showed that at least one of the comparisons involving the two factors studied (different treatments and different areas) was statistically significant ($F=43.78$; $p<0.001$). Tukey t-test for multiple comparisons was performed and the following results were found: (1) no statistical differences in the mean weight of eggplants were found between T2 and T3 within each area, suggesting no pollen insufficiency in the studied areas; (2) the mean weight of fruits formed from T2 differed significantly among areas, and the eggplants were heavier in the area IV (Ajapí: Organic). Such results suggest

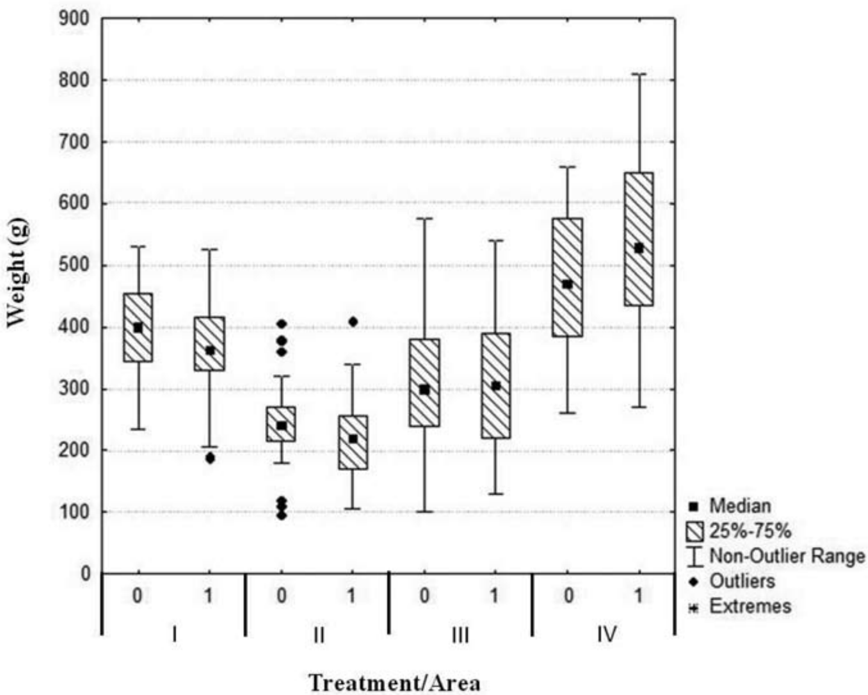

Fig. 2. Boxplots indicating the distribution of weights of eggplants collected for analyses. It is possible to visualize that within the same area no significant differences were found between the free visitation test (0) and pollen complementation test (1). However, a significant variation in the weight of eggplants was found when (1) was compared among areas I, II, III and IV.

that land management affects significantly the weight of eggplants and the pollination efficiency.

DISCUSSION

Many of the bee species collected in *S. melongena* flowers can be considered potential pollinators for this crop. In all of studied areas, we observed a predominance of bees capable of handling the anthers of eggplant flowers in a proper way, promoting the sonication of the anther's cone (King & Buchmann 2003) and the deposition of pollen grains on the surface of the stigma. Among them, large bees (Romero *et al.* 2011) such as those of *Bombus*, *Xylocopa*, *Centris*, *Epicharis* and *Oxaea* genus and small bees (Romero *et al.* 2011) such as *Exomalopsis* and many Halictidae species are included (Table 2).

The importance of these bee species to pollination of other species of *Solanum* was reported by different authors and is related to their ability to manipulate the poricid anthers, and also the large time spent in flower manipulation. Halictidae and bees of genus *Exomalopsis* are reported to vibrate each anther separately (Avanzi & Campos 1997; Forni-Martins *et al.* 1998; Bezerra & Machado 2003), but the simultaneous sonication of the entire anther cone, promoted by large bees, is considered to be more effective (Carvalho *et al.* 2001; Bezerra & Machado 2003; Gomig *et al.* 2007).

Despite *A. mellifera* and bees of genus *Trigona* being considered thieves of pollen and not very effective in eggplant pollination, the described behavior of *A. mellifera*, which flies up and down, very close to the flower, waving the anthers, can be somewhat effective in eggplant pollination. Amoako & Yeboa-Gyan (1991) reported that the pollination of tomatoes, peppers and eggplants by *A. mellifera* could increase the weight of fruits, if compared to those fruits formed without bee pollination.

Pollination tests emphasized the importance of bees for fruit setting in *S. melongena*. The exclusion of pollinator visits, by bagging flowers, induced flower drop and resulted in only a few fruits (Table 3), many of them, small and malformed (Fig. 1). Rylski *et al.* (1984), pointed out that some of the fruits observed in pollinator exclusion experiments could be produced by parthenocarpy and, in these cases, small and malformed fruits should be expected.

By contrast, the percentage of fruits formed from flowers, which received bee visitation (T2) or bee visitation plus pollen complementation (T3), was very high in all the four studied areas (Table 3). Gomig (2007) observed very similar results, but Montemor & Souza (2009) found a comparatively high percentage of fruit setting in flower bagging experiments (20%) and an unexpected low percentage of fruits formation in free visited flowers (40%). In this case, the authors didn't specify the length of stigma flowering during the tests.

S. melongena is a hetero-styled species (the flowers present short and long styles) and short-styled flowers rarely form fruits; on the other hand, about 90% of long-styled flowers result in well-formed fruits (Rylski *et al.* 1984; Kowalska, 2006). The use of short-styled flowers in the pollination tests could result in a low percentage of fruit set, even in free visitation or pollen complementation experiments.

Pollen complementation did not result in an increase in fruit setting success (Table 3) or in heavier fruits (Table 4, Fig. 2), which suggests that probably, there is no pollination insufficiency in the studied sites. The only exception occurred in area I, which pollen complementation resulted in an increase in the percentage of fruit setting success. In the other areas it was possible to observe a decrease in the percentage of fruit setting when extra pollen was deposited on stigma (T3). Stephenson (1981) reported that, in many plant species, a high level of pollination efficiency may cause an increase in fruit abortion rate, justified by the allocations of resources only to a few fruits, ensuring better fruit development and more viable seeds.

In Area I, the pollen complementation tests (T3) resulted in an increase in the percentage of fruit formation (100%), compared to the free insect visitation tests (T2, 84%), which could suggest that pollination services can be insufficient in this area. On the other hand, there was no significant difference in mean weight of the fruits formed in T2 *vs.*T3 (Table 4, Fig. 2), and the abundance of bumblebees in the area was very high - bees of genus *Bombus* are considered the most efficient pollinators of *S. melongena* (Aback *et al.* 1995; Kowalska 2006, 2008; Gemmill-Herren & Ochieng 2008; Montemor & Souza 2009).

Although the presence of effective pollinators in Area I suggests a higher level of pollination, pesticides, especially insecticides, were frequently used in this area, even during eggplant flowering periods. Pesticides can cause changes in foraging behavior of bees, by decreasing the visitation rate, the time spent in flowers or even the amount of transferred pollen grains. Behavioral changes such as those were described for honeybees and bumblebees with sub lethal doses of insecticides (Thompson & Hunt 1999; Bortolotti *et al.* 2003; Yang *et al.* 2008). Thompson (2003) found that the oral administration of insecticides induced errors in the wagtail dance in *Apis*, with consequent failure in the communication of the position of food sources. According to this author, insecticides can also repel bees from flowers and disturb their orientation.

Brittain *et al.* (2010) found that the successive application of pesticides in cropped areas was followed by a decrease in richness and abundance of bee species. Despite the intensive use of insecticides, Area I presented the more diversified fauna of visiting bees. To better understand the relationship between pesticide use, fauna diversity and pollination effectiveness, an investigation of the history of cleaning and other disturbances in the area will be necessary.

The mean weight of eggplant fruits formed from flowers that received free visitation of bees (T2) varied significantly among different areas (Table 4, Fig.2). Since in this study we tried to understand the effects of land management on the relationship between cropped areas and pollinator services, we did not interfere with agricultural practices in any of studied areas. Thus, many factors may be related to this high variability, such as the nutritional status of plants, pest control and soil irrigation.

Considering the importance of bees for fruit setting and the effects of the use of pesticides on bee foraging behavior, in this study, we emphasized the aspect of crop management.

Fruits formed in Area II presented the smaller mean weight, although no significant difference was observed when fruits formed in T2 were compared to those formed in T3 (Table 4; Fig. 2). During the period of this study, no pesticides were used; however, the use of insecticides in the crops, which occupied the area before eggplant cultivation, may have affected the bee fauna in that area.

Another aspect to be considered is the soil management. Despite the fact that the needs of eggplant crops in terms of soil nutrition are well known (Chen & Li 1996), no soil nutrition or pH corrections were observed in that area. The same soil management was observed in Area III. If the amount of nutrients necessary to supplement growth of eggplants was applied to this area, it would be possible to observe an increase in the mean weight of the fruits.

In Area IV, on the other hand, where cropping follows organic techniques and management, nutrients are provided by organic fertilization. Furthermore, the manager allows plants, which constitute pollen and nectar sources for bees, to grow around the planted area and provides nesting sites for bees, such as decayed wood, bare ground and bamboo clumps. These practices contribute to the maintenance of bees around cropped areas, even in periods when there is no flowering crop (Campos *et al.* 2006; Goulson *et al.* 2008). In this area, the percentage of the fruits formed in the T2 experiment was smaller (Table 2 - 82%) when compared to the other areas, however, the mean weight of the fruits was the highest, indicating a higher pollination efficiency (Table 4, Fig.2).

The relatively small percentage of fruit set in T2 in Area IV (Table 3) can be related to the presence of herbivores, such as *Diabrotica speciosa* (Coleoptera), which eats the stigmas and causes a great number of flower and fruit abortions. Kessler (2011) reported that in *Solanum peruvianum* (Solanaceae), volatile organic compounds, released after attack by herbivores, could repel other insects and interfere in pollinator-plant interactions and consequently on fruit setting. Another aspect to be considered is the presence of leaf cutting ants in the area; these ants cut off fruits in different development stages.

Since this is an organic farm, it is common to observe a greater diversity of insects associated with the crops. However, in Area IV, the manager commonly reaps the fruits 15 days after the opening of flowers, in contrast to the other areas, where the fruit harvest is performed at about 30 days after flower opening. The faster fruit development indicates that the area and soil management, together, can promote a more effective harvest and contributes to pollinator conservation.

CONCLUSION

The present study pointed out the importance of bees in promoting good yields in eggplant crops. Land management seems to be a factor that determines a better efficiency of pollination in agricultural landscapes, ensuring the supply of pollination services in cropped areas.

Good practices may enhance the establishment of viable populations of pollinators in the fields, but other practices, such as the continuous use of pesticides, can disrupt the crop–pollinator interactions by changing the composition of bee communities and the foraging behavior of bees, which may cause pollination insufficiency. Although there is research supporting that these changes are possible, most studies were conducted in laboratory conditions. So, it is still necessary to investigate the effects of pesticides on the foraging behavior of bees (duration of visits and flower manipulation behavior) under field conditions.

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