HONEY BEE AS AN EFFECTIVE POLLINATING AGENT OF PUMPKIN

Daniel Nicodemo[1]*; Regina Helena Nogueira Couto[1]; Euclides Braga Malheiros[2]; David De Jong[3]

[1]*UNESP/FCAV - Depto. de Zootecnia, Campus de Jaboticabal, Via de Acesso Prof. Paulo Donato Castellane s/n - 14884-900 - Jaboticabal, SP - Brasil.*
[2]*UNESP/FCAV - Depto. de Ciências Exatas.*
[3]*USP/FMRP - Depto. de Genética, Av. Bandeirantes, n° 3900 - 14049-900 - Ribeirão Preto, SP - Brasil.*
***Corresponding author <nicodemo@fcav.unesp.br>*

ABSTRACT: The production of fruits and seeds of many crops is increased when bees visit their flowers pollinating them. The aim of this research was to study the pollination of pumpkins (*Cucurbita maxima* Duch. var. Exposição), to determine the diversity of insects visiting its flowers, the time and type of provision obtained and the effect of the visits on fruit set, fruit size and weight, and number of seeds. *Apis mellifera* L. accounted for 73.4% of the visits made by bees, collecting pollen during 34.5 s per flower and nectar in 43.9 s and 29.3 s from female and male flowers, respectively. *Trigona spinipes* (Fabr.) collected only nectar, during a mean time of 60.5 s per flower, and represented 26.6% of the visits by bees. *Diabrotica speciosa* (Germ.) only fed on the petals of the flower. When no insect visits occurred, there was no production of fruits. In the flowers with free visitation by insects, fruit set was 40%. The higher the number of visits, up to 16, by *A. mellifera* to female flowers, the greater was the fruit set, fruit size and weight, and number of seeds. In flowers visited by insects from the onset of anthesis until 9 a.m., fruit set was 35%. After 9 a.m., there was no fruit set, demonstrating the important role of *A. mellifera* as a pollinating agent of pumpkin, since it was the only insect visiting up to 9 a.m.
Key words: *Cucurbita maxima*, pollination, honey bee

ABELHA MELÍFERA COMO EFICIENTE AGENTE POLINIZADOR DE MORANGA

RESUMO: A produção de frutos e sementes de várias culturas é favorecida quando abelhas visitam suas flores, efetuando a polinização. O objetivo deste trabalho foi estudar a polinização em moranga (*Cucurbita maxima* Duch. var. Exposição), no que se refere à identificação dos insetos que visitam mais freqüentemente suas flores, o tempo e tipo de recurso floral coletado e o resultado das visitas destes insetos na frutificação, tamanho, peso e número de sementes dos frutos. *Apis mellifera* L. foi responsável por 73.4% das visitas realizadas por abelhas, coletando pólen em 34.5 s e néctar em 43.9 s e 29.3 s, respectivamente para as flores femininas e masculinas. *Trigona spinipes* (Fabr.) coletou apenas néctar, em tempo médio de 60.5 s, representando 26.6% das visitas realizadas por abelhas. *Diabrotica speciosa* (Germ.) somente se alimentou das pétalas da flor. Não houve produção de frutos quando não ocorreu visita de insetos. Nas flores com livre visitação de insetos a frutificação foi de 40%. Quanto maior o número, até 16, de visitas de *A. mellifera* nas flores femininas, maior a frutificação, tamanho, peso e número de sementes. A frutificação foi de 35% no tratamento em que as flores permaneceram disponíveis à visitação desde o início da antese até às 9h00. A partir deste horário não houve frutificação, evidenciando o importante papel da *A. mellifera* como agente polinizador da moranga, já que foi o único inseto visitante até às 9h00.
Palavras-chave: *Cucurbita maxima*, polinização, abelha africanizada

INTRODUCTION

The destruction of natural habitats has reduced the nidification sites of various pollinating agents, affecting drastically their population. Many plant species depend on these agents for their production. Moreover, intensive farming has not only increased the demand for pollinators but also negatively affected their density and diversity (Nogueira-Couto, 2002).

Growers in some regions of Brazil have suffered with the decline in the production of fruits, especially those that have crops close to an urban area (Boti et al., 2005). Considering the importance of pollination in the establishment and production of fruits in various crops and the reduction in the diversity and abundance of pollinating agents, it is necessary to evaluate the need of controlled pollination of crops dependent on pollinating agents (Fonseca et al., 2006).

In Brazil, there are few reports on the pollination of crops of major commercial importance, and pumpkin (*Cucurbita maxima* Duch.) is one of the main ones. Pumpkin originated in Central America (Hartmann et al., 1988) is of great economic interest, being used as food among humans, as energy source and as animal feed, as a source of Beta-carotene.

Apis mellifera L. bees are effective pollinating agents of cucurbits (Thompson et al., 1955; Whitaker & Davis, 1962; Hurd, 1964; Couto et al., 1990). In the USA, *Peponapis pruinosa* has been shown to be the most important pollinating agent of pumpkins (Flottum, 2000). *Bombus terrestris* (L.) is also an effective pollinating agent of pumpkins (Fuchs & Müller, 2004).

The objective of this study was to evaluate pollination in pumpkins, variety Exposição, to determine the density and diversity of pollinators foraging on flowers, their behavior on the flowers and their resultant effects in terms of fruit production.

MATERIAL AND METHODS

The experiments were carried with pumpkin (*C. maxima*), variety Exposição, in 2001 and 2002. A pumpkin crop was cultivated in Jaboticabal, State of São Paulo, Brazil (21°15'22''S, 48°18'58''W, altitude 595 m). The area has a temperate subtropical climate, and a mean annual temperature of 21°C. The mean annual rainfall is 1431 mm. The region is intensively farmed with sugar cane being the dominant crop. Forests are reduced and fragmented, and the closest to the experimental area is more than 3 km away. An apiary with 30 hives was installed near the experimental area.

Soil analysis was performed in 2002 and afterwards the soil was limed in accordance with the recommendations for fertilizing and liming of the State of São Paulo (Raij et al., 1996), which differed from the preceding year when no lime was applied. In both years 128 pumpkin plants were grown with a spacing 3 m between plants and rows. Due to the lack of rain, plants were watered weekly.

Frequency of visitations by the insects, from 6h00 to 18h00, was made through visual observation by counting every 50 min from the start of visitation, for 10 min each hour, at the experiment site, with four replicates during different periods of each year.

In 2001 and 2002, during the flowering period, the most frequent insects were collected and preserved for later identification. The time and type of harvest (nectar and/or pollen) were determined for the most frequently appearing insects, at different hours, with 30 replicates, on four days of each test.

To obtain fruit production with and without visits by insects, 20 female flowers were chosen randomly before anthesis and marked, where half were covered with nylon bags which impeded visits by insects and the other half left uncovered with open pollination by insects. In the test, the edges of the patch were avoided, and five replicates were carried out for a total of 100 flowers per test. To determine the pollinating effectiveness of *A. mellifera*, female flowers were covered in pre-anthesis and uncovered in anthesis to allow visits exclusively by this species at pre-established frequencies (two, four, eight and 16 visits). Twenty flowers (replicates) were used for each frequency (treatment) of visits. Therefore, 80 flowers were utilized (four levels of visits × 20 replicates per test. The time of each visit by *A. mellifera* bees was recorded. The visits were permitted between 8h00 and 10h00. In each plant, only one flower per branch received visits and the flowers were randomly chosen for the treatments in a completely randomized design. After each visit, the flowers were labeled, protected again and establishment of fruit evaluated five days after the visitation, when the development of the ovary or abscission of the flower was observed. Fruits were removed when they reached commercial size. The method used followed Danka et al. (1983) & Stanghellini et al. (1997).

In 2002, a test was carried out to determine the effectiveness of visits by *A. mellifera* at different hours. Female flowers were protected before anthesis and left unprotected after anthesis to allow visits by the bees at four different visitation periods. In the first treatment, the flowers were uncovered after anthesis and covered again for 9 h. In the second treatment, the flowers were uncovered at 10h00 and covered again at 11h00. In the third treatment, the flowers were uncovered for 13 h and left that way. In the fourth treatment, the flowers received free visitation during the whole day. Each treatment included 20 flowers (replicates), totaling 80 flowers for four levels of visits × 20 replicates.

Time of fruit formation was determined, which was from fruit bud to the mature fruit, observing the percentage of fruit set in the treatments in which the female flowers remained covered or uncovered, with ten replicates for each treatment for each test. The size of each fruit was also measured. Fruits were weighed and examined with respect to the number of seeds per fruit. A similar analysis was carried out relative to the number of visits by *A. mellifera* per flower.

The experimental design utilized was completely randomized. Spearman's correlation was used to determine if fruit size and weight and number of seeds correlated with the number of visits. Student's t test was utilized for the comparison of fruits derived from flowers with free visitation and those that received 16 visits. Data were processed with the SAS program (1993).

RESULTS AND DISCUSSION

During the two years, *A. mellifera*, *Diabrotica speciosa* (Germ.) and *Trigona spinipes* (Fabr.) were the most frequent insects on the flowers of pumpkin plants. The peak presence of *A. mellifera* occurred at 8h00, for *T. spinipes* from 9h00 to 10h00, and for *D. speciosa* from 14h00 to 17h00, with no overlapping of these peak hours (Figure 1). *A. mellifera* visited the flowers to collect nectar and pollen, *T. spinipes* to collect exclusively nectar, and *D. speciosa* to feed on the flower petals. Considering only the bees *A. mellifera* and *T. spinipes*, their visits represented respectively 67.6% and 32.4% for the first year and 79.2% and 20.8% for the second year. *A. mellifera, T. spinipes* and *D. speciosa* were the insects most frequently found on pumpkin flowers in both years. A greater number of visits occurred early in the day with higher temperatures, but on colder days the visitation period lasted longer (Table 1).

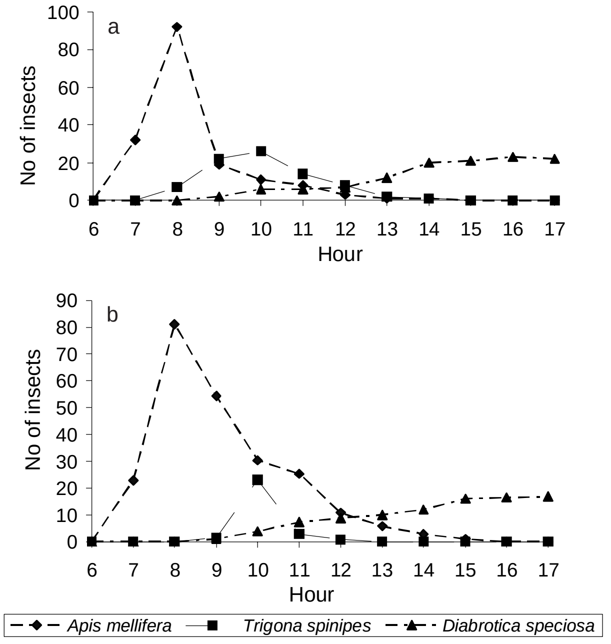

Figure 1 - Most frequent insects found on flowers of pumpkin (*Cucurbita maxima*), at different hours in 2001 (A) and 2002 (B).

The time of collection spent on each flower was different between years and between male and female flowers. The mean time of visit for *A. mellifera* per flower was 36 ± 5.6 s and 20 ± 4.4 s in 2001 and 51.8 ± 7.5 s and 38.6 ± 3.9 s in 2002, respectively for the harvest of nectar in female and male flowers, indicating that these bees spent more time collecting nectar in the female flowers. Generally, in diclinous monoecious plants, the female flowers produce more nectar than the male flowers (Nicodemo et al., 2007). It is possible that this is a strategy to attract insects since the male flowers offer pollen besides nectar. For the polen harvest, the results were not different between years. The time of visit of *A. mellifera* was 36.0 ± 8.1 s and 33.0 ± 4.6 s, respectively for the first and second years. *T. spinipes* collected only nectar in a mean time of 58 and 63 s, respectively in 2001 and 2002. *D. speciosa* harvested neither nectar nor pollen, and when it was on the female flower it did not come in contact with the stigma due to its small size, feeding only on the flower petals.

The time of fruit formation was 53 and 65 days after blooming respectively for the first and second year. The fruit was harvested when it presented the characteristic orange color and dry peduncle. When no visits by insects occurred on the flowers, there was no production of fruits (Table 2). With free visitation, fruit production was 38 and 42% in 2001 and 2002, respectively. In *Cucurbita pepo*, there is no production of fruits without visitation of flowers by insects (Couto et al., 1990).

With a greater number of visits by *A. mellifera* on the flowers, fruit set was increased, up to 16 visits per female flower. In the first year, the width of the fruits was greater in those from flowers that received a greater number of visits by *A. mellifera*, but there was no difference for the other observed parameters (Table 3). In the second year, there was a difference for length, width, number of seeds and mainly weight of the fruits (r = 0.85), where such characteristics benefited from the greater number of visits by *A. mellifera*. The resultant fruits of flowers that received 16 visits had double the weight of fruits resulting from flowers that were visited twice. Apparently, not only the number of visits is important for fruit production but also the time and type of harvest, because the longer the visit, the greater the chance of the insect coming in contact with the stigma of the flower.

Table 1 - Mean temperature of the days when insects were observed visiting the flowers of pumpkin.

Year	2001				2002			
Month	August				July			
Days	18	19	25	26	23	24	25	26
Mean temperature (°C)	19.8	20.2	21.0	21.3	19.9	20.2	20.5	21.1

Table 2 - Resulting fruit set of pumpkin from 50 flowers covered and 50 flowers with free visitation, in 2001 and 2002.

Treatment		Fruit set	Length	Width	Weight	No. of seeds
		%	----------- mm	-----------	g	
2001	Covered	0	-	-	-	-
	Uncovered	38	172.1	86.66	1133.7	130
2002	Covered	0	-	-	-	-
	Uncovered	42	181.8	101.9	1727.4	162.81

Table 3 - Fruit set of pumpkin resulting from flowers that received 2, 4, 8 and 16 visits by *Apis mellifera*, (80 flowers), time of visit, Spearman's correlation coefficient (r) and *p* value, in 2001 and 2002.

Treatment	Fruit set	Length	Width	Weight	No. of seeds	Mean time of visit
	%	-------------- mm	--------------	g		s
			2001			
2 visits	5	148.2	84.1	820	148.0	43
4 visits	5	163.9	89.6	1.140	184.0	41
8 visits	15	161.9	87.5	1.087	207.3	35
16 visits	55	160.7	98.1	1.202	188.2	34
r	-	0.07	0.61	0.23	0.61	-
p	-	> 0.20	0.01	> 0.20	> 0.81	-
			2002			
2 visits	15	152.3	93.7	963.3	82.3	48
4 visits	15	162.0	108.0	1086.0	106.7	43
8 visits	25	184.2	110.4	1613.0	143.6	36
16 visits	45	188.0	115.8	1932.9	175.1	30
r	-	0.71	0.51	0.85	0.76	-
p	-	< 0.01	0.02	< 0.01	< 0.01	-

The mean weight of all the fruits in the second year was greater than that for the first year, possibly due to the liming and also because the plants lasted 12 days longer than in the preceding year. As fertilizer conditions were better on the second year, the fruits became larger than the fruits from 2001 and for that the plants needed more time. In the two years, fruit set for the treatment with free visitation was less than for the treatment with 16 visits. This occurred because there could have been less than or more than 16 visits with visitation. Considering the possibility of more than 16 visits, there is some indication that excessive visitation can lead to the removal of pollen grains already deposited on the stigma of the flowers. With pollination, up to 74.6% fruit set can be obtained in pumpkins, where the climatic conditions are secondary when compared to the importance of the bees in fruit establishment (Stapleton et al., 2000).

The fruits from flowers that had 16 visits had a greater width (p = 0.04) and number of seeds (p = 0.01) in 2001 than the fruit resulting from flowers with free visitation which in turn had greater width (p = 0.03) in 2002, based on Student's t test. There was no difference for the other comparisons between these two treatments. Walters & Taylor (2006) studied the pollination of *Cucurbita pepo*, *C. moschata* and C. *maxima* and concluded that despite the presence of sufficient numbers of pollinating insects of pumpkin at the experiment location, fruit set, fruit size and weight and number of seed was greater when hives of *A. mellifera* were installed among the crops. Walters & Taylor (2006) noted that since pumpkins are sold by weight, the grower's earnings could be greater when *A. mellifera* hives are installed during the flowering of pumpkin plants. To know that, it is necessary to estimate the costs to rent honey bee colonies for pumpkin pollination.

The flowers open to visitation until 9h00 originated fruits with 35% fruit set, 171.14 mm in length, 100.29 mm in width, weight of 1,340 g and 120.57 seeds. However, the flowers open from 10h00 to 11h00 and from 13h00, did not give rise to any fruit. These findings demonstrate the efficacy of *A. mellifera* as a pollinating agent of pumpkin, since it is the only insect that visited the flowers until 9 h.

CONCLUSIONS

The most important insect seen on flowers of pumpkin was *A. mellifera*, which harvested nectar and pollen. *T. spinipes* harvested nectar and *D. speciosa* sucked the sap from the petals. Both did not pollinate pumpkin flowers. *A. mellifera* is an effective pollinating agent of the pumpkin crop. Fruit production occurs only when the insects visit the flowers up to 9 h. Fruit set, fruit size and weight and number of seed increased as the number of visits by *A. mellifera* also increased up to 16 visits per female flower, at which the highest fruit set level was reached.

ACKNOWLEDGMENTS

To FAPESP and CNPq, for the Scholarship and other support.

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Received December 17, 2007
Accepted December 15, 2008