Seed production and germination of sunflower (*Helianthus annuus* L.) in three pollination systems

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ABSTRACT. This study was carried out to evaluate seed production and germination of sunflower, *Helianthus annuus*. The sunflower cultivar used is called Embrapa 122-V2000. The experiment used a marked area that had 8m x 3m, and in a second area was installed, containing a honeybee colony and covered with wire screen, 8m x 3m, with two meters in the highest part. The seed production was 362.34% higher in the covered area with honeybee colony, and its achenes weight, 27.12% higher. Therefore, pollination by honeybees in sunflower increases the seed production, as well as its germination.

Key words: africanized honeybees, yield increasing, entomophilous pollination, production, seeds germination.

RESUMO. Produção de sementes de girassol (*Helianthus annuus* L.) em três sistemas de polinização. Esse experimento foi realizado para avaliar a produção e a germinação de sementes de girassol (*Helianthus annuus*). Foi utilizada uma área somente demarcada de 8m x 3m, e o desenvolvimento da colônia dentro de uma gaiola telada com 8m x 3m com dois metros de altura na sua porção mais alta. Entretanto, a produção de sementes foi 362,34% maior na área coberta com abelhas *Apis mellifera,* bem como 27,12%% no peso dos aquênios. Pode-se concluir que a polinização por abelhas no girassol proporcionou não só um aumento na produção de sementes, como também na sua viabilidade em relação à área coberta sem abelhas.

Palavras-chave: abelhas africanizadas, aumento de produção, polinização entomófila, produção, germinação de sementes.

Introduction

The sunflower cultivation area seems to be increasing every year and a pollination program with insects is necessary. Pollination is an important factor for the satisfactory development of the achenes and formation of viable seeds (McGregor, 1976). McGregor (1987) reported that the production of sunflower seeds needs pollination made by insects. Strong honeybee colonies should be used in the beginning of the flowering. Colonies should be well dispersed by the plantation so that the necessary saturation is supplied for the pollination. In some parts of the world like Russia, South Africa and Australia researchers recommend a beehive per hectare, while in Romania the recommendation is about two per hectare. A larger concentration of honeybees can be necessary for the production of hybrid seeds (Free, 1993).

Sunflower is the second largest source of eatable oil in the world. Its pie or bran is of great nutritional value for animals, especially dairy cattle, and is also indicated for silage, when cut at flourishing time and turned into bread-making flour (Pelegrini, 1985). Soares (1982) reported an increase in the sunflower productivity about 98.4%, due to the pollination made by honeybees.

Experiments with pollination, developed in Brazil, demonstrated that it is possible to increase the production of several crops. Also, Moreti (1989) reported an increase of 82% in sunflower seeds in var. Anhandy, 81% in var. Uruguay and 15% in var. Contisol, due to honeybee pollination.

Accomplishing works to verify the insect efficiency (*A. mellifera*, *Bombus terrestris* and *Megachile rotundata* or *Calliphora* ssp) in pollination of three sunflower species (*H. annuus*, *H. argophyllus*, and *H. debilis*), within cages and compared to manual pollination and free pollination, concluded that pollination with insects presented a higher percentage of seeds than the manual one (Meynie, 1995). For *H. debilis,* the pollination efficiency was the same for the four insect species tested. The most efficient pollinator for *H. annuus* and *H. argophyllus* was *B. terrestris*, followed by *A. mellifera*.

Moreti (1989), studying the efficiency of pollination by insects in sunflower, verified that a plant freely visited by honeybees showed a higher percentage of achenes formation, achenes weight, and

weight of 100 achenes, when compared to protected plants.

In *Pindamonhangaba* (State of *São Paulo*, southeastern Brazil), Moreti *et al.* (1993) observed the action of honeybees and other pollinator insects in sunflower (*H. annuus* L.), comparing three treatments: uncovered areas (free for insect visitation), cages containing a honeybee colony (flowers visited only by honeybees) and cages without honeybee colony (flowers not visited by insects). An increase of 47.38% and 67.39% was observed in the number and weight of seeds, respectively, although these results were not statistically different.

In *Viamão* (State of *Rio Grande do Sul*, southern Brazil), sunflower flowers visited by insects developed 52%. 96% of the visitors were *A. mellifera* (Hoffmann, 1994).

This experiment was carried out to evaluate the production of sunflower seeds and the efficiency of the pollination by insects in three systems: area not visited by insects, area only visited by honeybees and uncovered area freely visited by insects.

Material and methods

The experiment was conducted at *Fazenda Experimental de Iguatemi* (FEI) of the *Universidade Estadual de Maringá* (UEM), Brazil, located in the State of *Paraná*'s northwest area, with an annual mean temperature of 21.9ºC, and 554m of altitude (source: Department of Meteorology/UEM). The sunflower variety (*H. annuus*, L.) was developed by Soybean National Research Center, part of the Brazilian Agricultural Research Agency (Embrapa Soja). The variety is totally Brazilian, has dark-peeled achenes and is called Embrapa 122-V2000.

The study was developed from October 1999 to February 2000, in a total area of 1986m^2, inside which three areas of eight per three meters were demarcated (24m^2 each), with three replications each. Two pollination cages were built with plastic screen with a 2-mm mesh, which allows 80% brightness passage. The treatments consisted in: 1) a demarcated area, uncovered and freely visited by insects; 2) a covered area, containing a honeybee colony (*A. mellifera*) - area visited only by honeybees; and 3) a covered area without honeybees - area not visited by insects.

The cages were mounted some days before the flowering and were dismounted at the end of the flowering, to allow the complete vegetative development of the plants. The sunflower crop was evaluated daily during the whole flowering phase, until the ripening of the seeds.

The sunflower heads were let to dry freely in the atmosphere, separated by treatment and the diameters were measured. After the measurement of the sunflower heads, the achenes were cut for counting, and five 100-achenes samples per treatment were weighed individually in analytic scale (100 achenes per treatment).

The weight and the germination test of the seeds were accomplished in the laboratory of seeds analysis at FEI-UEM.

The germination test was performed according to the rules for seed analysis test established by the Ministry of Agriculture (Brazil, 1986). A total of 400 achenes were separated per treatment, and divided in groups of 50 (eight replications), settled in this work, in sheets of germitest paper as substratum. Achenes and paper were humidified and placed in an incubator at 30ºC. After three days, the number of germinated seeds was counted and removed and other were placed again in the incubator. After seven days, the test was interrupted and the last germinated seeds were counted. The number of germinated seeds was subtracted from the total of achenes, obtaining the number of non-germinated seeds.

The data were statistically analyzed using a completely randomized design. After the variance analysis, the averages were compared, using the Tukey's test (Pimentel Gomes, 1990), using the GLM procedure of S.A.S. (Sas Institute, 1998).

Results and discussion

The mean of the largest, smallest and medium diameters of the sunflower heads (cm), the mean weight of each achene (mg), the weight of one hundred achenes (g), total achenes weight for sunflower head (g) and achenes number for sunflower heads, by treatment, are in Table 1.

Table 1. F values with respective probability (P), coefficient of variation (CV) and means, followed by its standard deviation of the largest, smallest and medium diameters of the sunflower heads (cm), mean weight of achene (mg), one hundred achenes weight (g), total weight of achenes per sunflower head (g) and number of achenes per sunflower head

Variation source	Largest diameter of the sunflower heads (cm)	Smallest diameter of the sunflower heads (cm)	Medium diameter of the sunflower heads (cm)	Mean weight of achene (mg)	One hundred achenes weight (g)	Total weight of achenes per sunflower head (g)	Number of achenes per sunflower head
Treatment	89.78 P=0.0001	91.85 P=0.0001	94.27 P=0.0001	178.41 P=0.0001	288.68P=0.0001	71.49 P=0.0001	71.53 P=0.0001
C.V. (%)	23.36	23.66	23.06	32.02	4.82	75.19	62.84
Means Uncovered area	10.69 ± 2.22 a[1]	9.77 ± 2.11 a	10.23 ± 2.13 a	48.49 ± 12.81 a	6.19 ± 0.22 a	19.10 ± 10.73 a	385.44 ± 191.57 a

Covered area with honeybee colony	5.76 ± 1.57 b	5.07 ± 1.24 b	5.42 ± 1.37 b	19.62 ± 6.34 b	2.81 ± 0.29 c	2.25 ± 1.70 b	115.03 ± 73.68 b
Covered area without honeybee colony	5.94 ± 1.74 b	5.52 ± 1.43 b	5.73 ± 1.44 b	48.16 ± 16.05 a	5.18 ± 0.15 b	1.77 ± 2.03 b	24.88 ± 28.40 c

[1] Means followed by different letters, in the same column, differ statistically by the Tukey's test (P<0.05)

The largest, smallest and medium diameters of sunflower heads were higher (p=0.0001) in the uncovered area than in the other treatments. There was no difference (p>0.05) between the covered area with honeybee colony and covered area without honeybee colony (Table 1). The smaller diameter of sunflower heads decreased in treatments covered with and without honeybee colony, and this can be attributed to the brightness reduction due to the use of screen. The sunflower is a plant considered to present positive heliotropism (Vranceanu, 1977) and to have its flowering period extended by the occurrence of cloudy weather (Castiglioni *et al.,* 1997).

In relation to the mean weight of each achene, there was no difference (p>0.05) between uncovered area and covered area without honeybee colony, but the covered area with honeybee colony presented larger achenes (p=0.0001) when compared to the other treatments. The uncovered area showed more weight for 100 achenes (p=0.0001) than the others. In the covered area with honeybee colony, the achenes had lower mean weight (p=0.0001) when compared to the other treatments.

In relation to the achenes total weight per sunflower head, the uncovered area showed better results, and there was no difference (p>0.05) between the other two covered areas.

Martins (2003) concluded that the honeybee colony is a more efficient sunflower pollinator when the colonies are located nearest the sunflower crop (about 33m). This experiment had no colony near the sunflower crop.

For the achenes number per sunflower head, the uncovered area produced more achenes than the one covered with honeybees, which, in turn, produced more than the covered area set without honeybees. The weight of 100 achenes is affected by quantity of seeds by head (Singh *et al.,* 2001), which explains the results found in this research.

The seed production of the uncovered system (mean of 385.44 achenes for sunflower head) was significantly higher (p=0.0001) when compared to the covered areas, with and without honeybees. This result agrees with Moreti (1989), who found an increase of 40%, Moreti *et al*. (1996), who found production above 75%, and Hoffmann (1994), who found an increase of 56% in achene production for pollination by insects. Even though Delaplane and Mayer (2000) reported that some sunflower plants have a primary flower head and one or more secondary heads, single-headed plants are the usual in commercial varieties. The primary head has 1000-4000 florets and the secondary, 500-1500 florets.

The production of seeds in sunflower was higher in the uncovered area, followed by the covered area with honeybee colony. Ahmed *et al*. (1989) obtained similar results working with the same systems in a smaller area. According to Fell (1986), most honeybees visiting sunflower are collecting nectar, not pollen. Free (1993) and Paiva *et al.* (2002) reported that honeybees collecting nectar are more important than honeybees collecting pollen for sunflower pollination.

The production of seeds in the uncovered area was 70.6% and 93.55%, compared to the covered area with honeybee colony and the covered area without honeybee colony, respectively. An increase of 78.37% was found in seed production in the covered area with honeybee colony, compared to covered area without honeybee colony. These data agree with Amaral (1970), who found a production of 8.85 seeds for sunflower head in the isolated system, 98.40% less than in the uncovered area and 95.21% less than in the covered area with honeybee colony, as well with Du Toit (1990), who found the production of seeds in cage with *A. mellifera* 38% higher than in cage without honeybees.

The germination test results in three and seven days for treatment are shown in Table 2. In three days, the uncovered area and the covered area with honeybee colony presented higher percentages (p=0.0001) than the covered area without honeybees. Those two treatments did not differ between each other (p>0.05). The germination test in 7 days did not show difference (p=0.0750) among the treatments.

The uncovered area for honeybee visitation had 22% more germination than the isolated area. This agrees with Moreti (1989), who found an increase of 30% in the area uncovered for insect visitation. It also agrees with Ahmed *et al.* (1989) who, in an experiment between 1985 and 1986, found an increase of 26.44% in the system free for visitation and 16.88% in the cage system with a colony of *A. mellifera*, in relation to the isolated system. The differences in the germination percentages can be attributed to the fact that researchers used different sunflower varieties than the one used in this experiment. The drought occurred between the plantation and the beginning of sunflower flowering could also have influenced the results of this experiment.

Table 2. F values with its respective probability (P), coefficient of variation (CV), and means of seeds germination of *Helianthus annuus* at three and seven days. Transformed data in arcsine $\sqrt{x+\alpha}$, $\alpha = 0.5$

Variation source	Seeds germination after three days	Seeds germination after seven days
Treatment	18.14 P=0.0001	2.94 P=0.075
CV (%)	10.18	23.63
Means Uncovered area	1.05 ± 0.08 a[1] (74.75 ± 6.76)[2]	0.28 ± 0.06 a (7.25 ± 3.01)
Covered area with honeybee colony	1.00 ± 0.12 a (69.75 ± 10.77)	0.26 ± 0.09 a (6.75 ± 5.12)
Covered area without honeybee colony	0.78 ± 0.08 b (49.00 ± 7.93)	0.34 ± 0.05 a (10.75 ± 3.01)

[1] mean followed by different letters, in the same column, differ statistically by the Tukey's test (P<0.05); [2] data inside parenthesis are means plus standard deviation without transformation

There were changes in the sunflower development, due to the small rain precipitation occurred in the end of 1999, totaling 86.8 millimeters between plantation time (October 20, 1999) and flowering time (December 21, 1999). Plants began flowering 63 days after the plantation, reaching a height of 110cm. According to the botanical characteristic of cultivating, indicated by Embrapa Soja, the beginning of the flowering would be at 53 days and the plants should reach the height of 155cm. The nutrients repartition inside the plant was affected by this environmental factor, affecting thus the nectar production and the insect visitation on the sunflower flower. This factor affected all parameters evaluated, too.

Although the mean weight of each achene and the weight of 100 achenes in the covered area with honeybee colony were inferior to the uncovered area and the covered area without honeybee colony (59.3% and 45.8%, respectively), the uncovered area had higher values than the covered area without honeybee colony in the germination test (17.6%) and for the achenes number per sunflower head (78.37%).

These facts demonstrate that pollination by honeybees provided a greater production and germination of achenes for the covered area with honeybee colony, when compared to the covered area without honeybee colony.

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