Vol.48, n. 1 : pp. 31-36, January 2005 ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY

AN INTERNATIONAL JOURNAL

Pollination of Soybean (Glycine max L. Merril) by Honeybees (Apis mellifera L.)

Wainer César Chiari¹, Vagner de Alencar Arnaut de Toledo^{1*}, Maria Claudia Colla Ruvolo-Takasusuki², Arildo José Braz de Oliveira³, Eduardo Shiguero Sakaguti¹, Valeria Maria Attencia¹, Fabiana Martins Costa¹ and Marina Hitomi Mitsui¹

¹ Departamento de Zootecnia; vaatoledo@uem.br; ² Departamento de Biologia Celular e Genética; ³ Departamento de Farmácia; Universidade Estadual de Maringá - UEM; Av. Colombo, 5790; 87.020-900; Maringá - PR - Brazil

ABSTRACT

This experiment was carried out to evaluate the effect of the honeybee pollination in the production and quality of soybean seeds (Glycine max L. Merril). Seed production was higher (P=0.0001) in covered areas with honeybee colonies (50.64%) and uncovered areas (57.73%) than in covered areas without honeybee colonies. It could be concluded that honeybees were responsible for 95.5% of the pollination accomplished by insects. The pod number in covered treatment with honeybees was 61.38% higher (P=0.0002) than in the covered treatment without honeybees. The average weight of 100 seeds was larger (P=0.0001) in the area covered without honeybees, and reached 17.8 g. The medium content of crude protein in grains was 36.7% and the average oil content was 20.2%. The germination test did not show differences (P>0.05) among the seeds in different treatments. It was concluded that the honeybee pollination in the soybean increased the seeds production.

Key words: Apis mellifera, soybean, Glycine max, honeybees, pollination, seed production

INTRODUCTION

The soybean (*Glycine max* L. Merril) is one of the most cultivated grains in the entire world. In the United States the crop had an estimated value of US\$ 16,490,700,000.00 in 1998 (Morse and Calderone, 2000). About 10% of this production was by pollination made by insects and 50% by *Apis mellifera* L. The estimated benefit was US\$ 824,500,000.00 to the agriculture. Soybean flower structure ensures to the honeybees the harvesting, favoring the pollen transference and the production increasing (Erickson and Garment, 1979). Other authors like Morse and Carter (1937), Rubis (1970) considered the soybean like autogamic.

Self-pollination can occur in some plants, without the necessity of the action of pollinator, while other needs to receive pollen of other plants of the same species. However, auto-fertility species can benefit from cross-pollination, ensuring higher productions (Crane and Walker, 1983). Paiva (2000) observed in experiments with sunflowers (*Helianthus annuus*) increase in the seeds production (78.37%) when compared with plants without access to the pollination made by insects. Juliano (1976) reported in the pollinated soybean, increase the pods number (37.95%), and the pods average weight (39.85%) related to the non pollinated (control), to the Santa Rosa variety. According to Issa et al. (1984), the honeybee is an

According to Issa et al. (1984), the honeybee is an efficient pollinator for some soybean varieties

_

^{*} Author for correspondence

which resulted an increase in the seed production (95%) and 81% to the varieties IAC-5115 and IAC-3, respectively under the same conditions. The soybean plants, v. IAC-114 showed increase in the pods number (58.58%) and seeds (82.31%)when visited by the honeybees (Moreti et al., 1998). Erickson (1975), Abrams et al. (1978) and Erickson et al. (1978), working with G. max, found an increase of 5 to 20% in the soybean production with the colony collocation of A. mellifera in experiments with cages. Pollination studies of legume forages showed that in the culture of perennial soybean (G. wightii) there was an increase in the pod production (55.8%) and seeds (44.7%) in the presence of A. mellifera (Nogueira and Pereira, 1983; Nogueira-Couto et al., 1998). This study was carried out to evaluate the production and quality of seeds obtained in different pollination systems in soybean (Glycine max Merril) culture, variety BRS-133, and lot 710B in Maringá-Paraná, Brazil.

MATERIAL AND METHODS

The experiment was carried out in a 15 ha field of soybeans variety BRS-133. Pollination cages were made of nylon screen of 2 x 2 mm, supported by pipes of $\frac{1}{4}$ of inch in PVC, forming cages of four meters width, six meters length and two meters height in the highest part, on an area of 24 m^2 (Fig. 1).

The soybean crop was harvested after 122 days and was monitored with particular attention during the blooming. The treatments were: (a)- uncovered areas, 24 m² each, demarcated with colored ribbon; (b)- covered area with honeybees, one colony of five combs and (c)- covered area without honeybees. Each treatment replicated five times. The areas chosen for study were demarcated at random in the soybean field and the cages were put immediately before the beginning of blossom and when the last blossom closed.

The seed production was obtained through the clean, classification and weight of grains collected in the central area of each plot (12 m²) in all treatments. Samples were collected at random from 35 soybean plants in each plot to obtain the average of number of pods and seeds in each pod in each harvested plant of the three treatments. The evaluation of average weight of seeds was made through weights of 100 seeds (five replications). The germination test of the seeds was made as

described in Ministério da Agricultura (Brasil, 1986). The crude protein analysis and ether extract of the soybean grains were made according to the methods of Silva (1990).

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



Figure 1 - Pollination cage model used in the experiment with dimensions 4 m x 6 m.

RESULTS

Table 1 shows production in the experimental area (production/plant (g), production/ha (kg) and sacs/ha in different treatments). There was no difference (P>0.05) among the uncovered area and covered area with honeybees. However, in (P=0.0001) covered area without honeybees. Estimated production was 2,394.58 kg/ha or 39.91 sacs/ha. The production in covered area with honeybees was 50.64% higher than in covered area without honeybees or, 20.21 sacs/ha more.

In the opened area, freely visited by insects, the increase in the seed production related to the treatment covered without honeybees was 57.73%, or 23.04 sacs/ha. The pods and seeds number in uncovered area, covered with honeybees and covered without honeybees, presented differences between themselves (P=0.0001) (Table 2).

The pod number in covered area with honeybees was 61.38% higher (P=0.0002) than in the covered area without honeybees, and this was 90.71% smaller than observed in free treatment. In areas where *A. mellifera* was responsible for the pollination there was an increase of 58.86% in the

number of seeds in comparison to the treatment in that pollination was not allowed. It could be observed from Table 3 that the average weight of 100 seeds did not differ between the treatment uncovered and covered with honeybees (P>0.05), however, these treatments were inferior to the covered without honeybees (P=0.0001).

The results of the seeds germination test in three treatments are presented in Table 4. The germination percentage did not differ (P>0.05) between the treatments. Meanwhile, the normal

plant number was superior (P=0.0001) in uncovered area, in relation to the treatments covered with honeybees and covered without honeybees.

Table 5 shows the results of bromatological analysis of seeds sampled in three treatments. There was no difference (P>0.05) between the treatments. Crude protein content and ether extract were in the normal rate to the soybean grain. The average content of crude protein in the seeds was $36.69 \pm 1.08\%$ and of ether extracts $20.24 \pm 1.12\%$.

Table 1 - F values with respective probability (P), coefficient of variation (CV%), production (g) of 12 m², production (g/plant), production in kg/ha and production of sacs/ha of soybean *Glycine max*, variety BRS-113

Variation source	Production in 12 m ² (g)	Production/ha (kg)	Production (sacs/ha)
Treatments	100.05 P=0.0001	100.05 P=0.0001	100.05 P=0.0001
CV % Uncovered area	3.45 4532.40 a (±169.71)	3.45 3777.00 a (±141.43)	3.45 62.95 a (± 2.37)
Covered area with honeybees	4328.60 a (±133.40)	3607.17 a (±111.17)	60.12 a (± 1.85)
Covered area without honeybees	2873.50 b (± 80.00)	2394.58 b (± 65.00)	39.91 b (± 2.00)

Averages followed by different small letters, in the same column, are different by Tukey's test (P<0.05)

DISCUSSION

There are a few studies on pollination in soybean. For example Erickson (1975, 1984) found an increase from 5-20% and Issa et al. (1984) from 9-81% for two varieties IAC-5115 and IAC-3, respectively.

The results found in own experiment corresponded to a reduction of 4.5% in the area with free visits in relation to the covered area with honeybees. These results suggested that the pollination made by insects, in particular *A. mellifera*, was responsible for the increase in the productivity.

In the treatments with pollination made by honeybees *A. mellifera*, the average increase in the pods number was 61.38% and of seeds 58.86%. These results are similar to these found by Moreti et al. (1998), 58.58% in the pods number and 82.31% in the seeds number and by Nogueira-Couto and Pereira (1983) and

Nogueira-Couto et al. (1998), 55.8% in pod number. But these results are superior to the ones found by Erickson (1975) and Erickson et al. (1978) that obtained income between 5 and 20% in the seeds number and Juliano (1976) that found in variety Santa Rosa an increase of 37.95% in the pod numbers. Increasing in the seeds number in covered area with honeybees and uncovered area in relation to the place setting without honeybees were not followed by the increase in average weight of the seeds.

Table 2 - F values with the respective probability (P), coefficient of variation (CV%) of average number of pods and seeds counted from samples of plants of soybean *Glycine max*, variety BRS-133

Variation source	Number of pods	Number of seeds
Treatments	9.98	9.58
	P=0.0002	P = 0.0002
CV%	30.06	30.40
Uncovered area	49.87 a*	111.01 a
	(± 13.00)	(± 28.71)
Covered with honeybees	40.83 b	90.71 b
-	(± 13.88)	(± 31.90)
Covered without honeybees	25.30 c	57.10 c
•	(± 7.69)	(± 15.74)

Averages followed by different small letters, in the same column, are different by Tukey's test (P<0.05)

Table 3 - F values with respective probability (P), coefficient of variation (CV%) and weight of 100 seeds in (g) of soybean *Glycine max*, variety BRS-133

Variation source	Weight of 100 seeds (g)	
Treatments	33.65 P= 0.0001	
CV%	5.27	
Uncovered area	15.26 b (± 0.95)	
Covered with honeybees	15.37 b (± 0.64)	
Covered without honeybees	17.80 a (± 0.91)	

Averages followed by different small letters, in the same column, are different by Tukey's test (P<0.05)

Table 4 - F values with the respective probability (P), coefficient of variation (CV%) and the percentage of normal plants, abnormal plants, dead and the percentage of soybean *Glycine max* seeds germination, BRS-113 variety

Variation source	Normal (%)	Abnormal (%)	Germination (%)
Treatments	10.25 P=0.0001	8.42 P=0.0005	2.31 P=0.1052
CV%	5.25	14.87	9.28
Uncovered area	$0.68 \pm 0.30 \; a^*$	0.27 ± 0.41 b	1.32 ± 0.12
	$(39.30 \pm 2.98)**$	(7.10 ± 2.09)	(92.80 ± 4.72)
Covered with	0.64 ± 0.40 b	0.28 ± 0.40 a	1.27 ± 0.13
honeybees	(36.13 ± 3.90)	(8.75 ± 2.40)	(89.75 ± 6.33)
Covered without	0.63 ± 0.31 b	0.32 ± 0.34 a	1.27 ± 0.09
honeybees	(35.13 ± 3.73)	(10.13 ± 2.10)	(90.50 ± 4.75)

^{*} Mean followed by different small letters, in the same column, are different by Tukey's test (P<0.05)

Table 5 - F values with respective probability (P), coefficient of variation (CV%) and the percentage of protein and ether extract in the soybean seeds *Glycine max*, variety BRS-133

Variation source	Protein	Etherl extract
Treatments	0.73 P=0.5124	0.13 P=0.8828
CV%	3.08	6.09
Uncovered area	36.24 (± 1.30)	$20.22 (\pm 1.70)$
Covered with honeybees	$37.01 (\pm 0.93)$	$20.14 (\pm 0.40)$
Covered without honeybees	37.27 (± 0.89)	$20.82 (\pm 0.86)$

^{* *} Numbers between parenthesis show the not transformed means and the standard error

These seeds were 13.96% heavier in covered area without honeybees, when compared with other treatments. These results disagree with Levin (1983) and Malerbo-Souza (1996) that reported seeds and fruits larger and heavier, with more sweetened flavor, when resulting of crosspollination made by insects, but they are in agreement to Fávaro and Nogueira-Couto (2000) that found an increase of 15.45% in the medium weight of the seeds in the treatment in which the plants did not receive visitation of insects.

The largest weight found in seeds originated by auto-pollination could be consequence of a larger amount of nutrients available for their development. Like this, the total weight of a high number of smaller seeds was superior to the total weight of bigger seeds in small number. This fact suggested that could be an excess of nutrients in the plants of the covered area without honeybees, as because of the lack of pollination and decrease in the fertilization, it would not be possible to use these recourses that result in low productivity.

There are soybean varieties that can be benefited by the insects through the pollination (Erickson,1975, 1984; Issa et al., 1984; Moreti et al., 1998), increasing the productivity.

The *A. mellifera* honeybees were efficient to accomplish the cross-pollination works in the soybean flower and their uses in the agriculture bring considerable gain. The utilization of *A. mellifera* as pollinators in the soybean culture cultivated in big areas is not common in Brazil, but the beehive demand is big.

ACKNOWLEDGEMENTS

We are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) process no. 479868/01-8 for financial support and Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) for the grant of scholarships.

RESUMO

Este experimento teve como objetivo avaliar a polinização realizada pelas abelhas na produção e qualidade das sementes da soja (*Glycine max* L. Merril) na região de Maringá-PR. Os tratamentos constituíram de áreas demarcadas de livre

visitação por insetos, áreas cobertas por gaiolas com uma colônia de abelhas (Apis mellifera) e plantas também cobertas por gaiolas que impediam a visitação por insetos. Todas as áreas possuíam 24 m² (4 m x 6 m), com cinco repetições cada. A produção de sementes foi maior (P=0,0001) nas áreas cobertas com abelhas e de livre visitação com um incremento produtividade 50.64% de e 57.73%. respectivamente, em relação à área coberta sem abelhas. Pode-se considerar que as abelhas A. mellifera foram responsáveis por 95,5% da polinização realizada pelos insetos no tratamento livre. O número de vagens no tratamento coberto com abelhas foi 61,38% maior (P=0,0002) do que no coberto sem abelhas. Onde as abelhas A. mellifera foram responsáveis pela polinização cruzada, houve um aumento de 58,86% no número de sementes em relação ao tratamento onde não foi permitida a polinização realizada por insetos. Entretanto, o peso médio de 100 sementes foi maior (P=0,0001) na área coberta sem abelhas, atingiu um peso médio de 17,80 g, mostrando que plantas com menor produção formaram sementes maiores. No tratamento livre, o peso médio de 100 sementes foi de 15,26 g e no coberto com abelhas foi de 15,37 g. O teor médio de proteína bruta no grão foi de 36,69% e a média do teor de óleo foi de 20,24%. O teste de germinação não mostrou diferenças entre as sementes nos diferentes tratamentos. Pode-se concluir que as abelhas A. mellifera foram eficientes no trabalho polinização na soja, proporcionando um aumento considerável na produção de grãos e estes resultados reforcam a necessidade do uso das abelhas A. mellifera para elevar a produtividade da soja.

REFERENCES

Abrams, R. I.; Edwards, C. R. and Harris, T. (1978), Yields and cross-pollination of soybeans as affected by honeybees and alfalfa leaf cutting bees. *American Bee Journal*, **118**, 555-556, 558.

Brasil. (1986), Ministério da Agricultura. *Regras para análise de sementes*. Brasília: DNPV.

Crane, E. and Walker, P. (1983), Pollination of tropical and subtropical crops by bees. In: Crane, E., Walker, P. *The impact of pest management on bees and pollination*. Cardiff: IBRA. pp. 5-21.

Erickson, E. H. (1975), Effect of honey bees on yield of three soybean cultivars. *Crop Science*, **15**: (1), 84-86.

- Erickson, E. H. (1984), Soybean pollination and honey production a research progress report. *American Bee Journal*, **124**, 775-779.
- Erickson, E. H. and Garment, M. B. (1979), Soya-bean flowers: nectary ultra structure, nectar guides, and orientation on the flower by foraging honeybees. *Journal of Apicultural Research*, **18**: (1), 1-11.
- Erickson, E. H.; Berger, G. S. and Shannon, J. G. et al. (1978), Honey bee pollination increases soybean yields in the Mississippi Delta Region of Arkansas and Missouri. *Journal of Economic Entomology*, **71**, 601-603.
- Fávero, A. C. and Nogueira-Couto, R. H. (2000), Polinização em soja. In: Congresso Nacional de Apicultura, 13., Florianópolis SC. *Anais...* Florianópolis SC.
- Issa, M. R. C.; Velocci, M. E. P. and Gonçalves, L. S. et al. (1984), Ensaio de polinização da soja (*Glycine max*) por abelhas (*Apis mellifera*). In: Congresso Brasileiro de Apicultura, 5.; Congresso Ibero-Americano de Apicultura, 3., Viçosa MG. *Anais...* Viçosa MG.
- Juliano, J. C. (1976), Polinização entomófila da soja. In: Congresso Brasileiro de Apicultura, 4., Curitiba - PR. Anais... Curitiba - PR.
- Levin, M. D. (1983), Value of bee pollination to U.S. agriculture. *Bulletin of the Entomological Society of America*, **29**, 59-51.
- Malerbo-Souza, D. T. (1996), Efeitos de atrativos e repelentes sobre o comportamento forrageiro de abelhas, *Apis mellifera*. Tese (Doutorado) Faculdade de Ciências Agrárias e Veterinária de Jaboticabal, Jaboticabal SP, Brasil.
- Moreti, A. C. C. C.; Silva, E. C. A. and Alves, M. L. T. M. F. et al. (1998), Observações sobre a polinização entomófila da cultura da soja (*Glycine max Merril*). *Boletim da Indústria Animal*, **55**: (1), 91-94.
- Morse, R. A. and Cartter, J. L. (1937), Improvement in soybeans. *Yb. U.S. Dep. Agric.*, 1154-1159.
- Morse, R. A. and Calderone, N. W. (2000), The value of honey bees as pollinators of U.S. crops in 2000. *Bee Culture*, **132**: (3), 1-15.

- Nogueira-Couto, R. H. and Pereira, J. M. S. (1983), Polinização entomófila em *Glycine wightii* (soja perene). In: Reunião da SBPC, 35., Belém PA. *Anais.*.. Belém PA.
- Nogueira-Couto, R. H.; Pereira, J. M. S. and De Jong, D. (1998), Pollination of *Glycine wightii*, a perennial soybean, by Africanized honey bees. *Journal of Apicultural Research*, **3**: (4), 289-291.
- Paiva, G. J. (2000), Comparação da produção de sementes de girassol (*Helianthus annuus* L.) em três sistemas de polinização por abelhas. Dissertação (Mestrado), Universidade Estadual de Maringá, Maringá - PR, Brasil.
- Pimentel Gomes, F. (1990), *Curso de estatística experimental*. Piracicaba: Nobel.
- Rubis, D. D. (1970), Breeding insect pollinated crops. Arkansas Agricultural Extension Service, 127, 19-24.
- SAS Institute (1998), *User's guide*. Carry, NC: SAS Institute.
- Silva, D. J. (1990), Análise de alimentos: métodos químicos e biológicos. Viçosa-MG: UFV.

Received: August 06, 2003; Revised: December 11, 2003; Accepted: July 15, 2004.