http://dx.doi.org/10.1590/1678-4162-8875

***Arq. Bras. Med. Vet. Zootec.*, v.68, n.5, p.1369-1373, 2016**

# Differential performance of honey bee colonies selected for bee-pollen production through instrumental insemination and free-mating technique

[*Comparação do desempenho de colônias de abelhas melíferas selecionadas para maiores produções de pólen apícola, por meio de inseminação instrumental e acasalamento livre*]

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## ABSTRACT

The use of bee-pollen as a nutritional supplement or as a production-enhancing agent in livestock has increased the demand for this product worldwide. Despite the current importance of this niche within the apiculture industry, few studies have addressed the pollen production. We tested the performance of free-mated (FM) and instrumentally inseminated queens (IQ) in order to establish the effect of different breeding systems on pollen production. The F1 generation of IQ queens produced 153.95±42.83g/day, showing a significant improvement on the pollen production (2.74 times) when compared to the parental generation (51.83±7.84g/day). The F1 generation of free-mated queens produced 100.07±8.23 g/day, which increased by 1.78 times when compared to the parental generation. Furthermore, we observed a statistically significant difference between the pollen production between colonies from the IQ and FM treatments. This study suggests that inseminated queens should be considered by beekeepers that aim to increase pollen production.

Keywords: Apis mellifera, bee-pollen, instrumental insemination, pollen production, Africanized honey bees

## RESUMO

*O uso do pólen apícola como suplemento nutricional humano e animal tem aumentado significativamente a demanda por este produto em todo o mundo. Apesar da importância atual desse nicho dentro da apicultura, poucos estudos têm abordado melhorias no sistema produtivo de pólen. Testamos o desempenho de rainhas fecundadas através de acasalamento livre (FM) e inseminação instrumental (QI), a fim de analisar o efeito de diferentes técnicas de melhoramento genético no ganho produtivo de pólen apícola. A geração F1 de rainhas QI produziu 153,95±42,83g de pólen por dia, mostrando uma melhora significativa na produção (2,74 vezes) quando comparado à geração parental (51,83±7,84g/dia). A geração F1 de rainhas de livre acasalamento produziu 100,07±8,23g/dia, o que significou um aumento de 1,78 vezes. Além disso, observou-se uma diferença estatisticamente significativa entre a produção de pólen entre as colônias dos tratamentos QI e FM. Este estudo sugere que a técnica de inseminação instrumental deve ser incorporada nos sistemas produtivos de pólen apícola brasileiro.*

*Palavras-chave:* Apis mellifera, *pólen apícola, inseminação instrumental, produção de pólen, abelhas africanizadas*

## INTRODUCTION

The honey bee's pollination services are crucial to improve or produce 87 crops, that represent 70% of crops used directly for human consumption (Williams, 1994; Klein *et al*., 2007; Gallai *et al*., 2009). The pollen collected and transferred among different flowers (pollination) facilitates plant reproduction and, as a mutualistic relationship, provides bees the collected surplus pollen as a food resource. Because of its nutritional quality, bee-pollen has

Recebido em 12 de novembro de 2015
Aceito em 4 de março de 2016
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been incorporated into human diets. Its use has also been encouraged for potential benefits against diseases, especially prostatic cancer (Habib *et al*., 1990; Zhang *et al*., 1995; Hana *et al*., 2007; Wu and Lou, 2007; Wang *et al*., 2015), chronic prostatitis (Dhar and Shoskes, 2007; Buck *et al*., 1989), and prostatic hyperplasia (Xu *et al*., 2008). Athletes, ever chasing improved performance, are also encouraged to consume bee-pollen on a regular basis (Maughan and Evans, 1982). In non-human systems, bee-pollen has also been recommended as a productive-enhancing agent in a wide range of livestock, such as rabbits (Attia *et al*., 2011), horses (Turner *et al*., 2006), broiler chickens (Attia *et al*., 2014), and fish (El-Asely *et al*., 2014). These therapeutic and animal-feeding usages of bee-pollen have increased the demand for this product worldwide, which has consequently caused prices to rise. As such, bee-pollen production has become a very profitable beekeeping practice and has assumed a significant economic role in many parts of the world, especially Australia, Argentina, Brazil, China, Spain and Vietnam (Value…, 1996).

Despite the current importance of this niche within the apiculture industry, few studies have aimed to increase pollen collection behavior for production purposes. While much has been done to improve honey production (Szabo and Lefkovitch, 1988) and resistance against parasites and diseases (Rinderer *et al*., 2010; Rosenkranz *et al*., 2010), the pollen production system has not been improved in years, and many techniques have never been validated. Particularly in Brazil, there is a need for studies concerning the applicability of selective breeding in the pollen-productive system once the only methodology applied by beekeepers is based on unilateral selection of parental (queen rearing followed by free-mating). Instrumental insemination has never been part of a breeding program aiming for bee-pollen production in Brazil, so beekeepers remain reluctant to use inseminated queens. In this study, we registered the performance of free-mated (FM) and instrumentally inseminated queens (IQ), for the first time in Brazil, in order to establish what type of breeding system is more suitable to increase pollen foraging behavior in honey bees.

## MATERIAL AND METHODS

The study was conducted from 2013 to 2014 in the municipality of Ribeirão Preto, São Paulo State, Southeast Brazil (latitude: 21° 10' 42", longitude: 47° 48' 24", altitude: 545 m). The experimental apiary was surrounded by an *Eucalipstus sp* abandoned crop and native vegetation. We initiated the experiment by measuring pollen collections of 80 standard Langstroth hives (Pop). Those hives presented, approximately, a population size of 29,680 bees (Burgett and Barikam, 1985), compiling one brood chamber (8 brood frames covered with bees and 2 food frames), and a single super (10 food frames). Same-aged African-derived queens headed all colonies. Pollen traps were installed between the brood chamber and the bottom board. We collected pollen every 48 hours for one month, starting after the end of the first week. At the end of the sampling period, we calculated the average pollen collected (in grams) per 24 hours for each colony. Subsequently, the two colonies that presented the highest average pollen production were selected as the breeding stock (BS): Colony A and B. The other 78 were classified as the voluntary culling group (VC). A F1 generation of queens was produced from the BS colonies following standard queen rearing practices (Laidlaw and Page, 1997). This F1 queen generation was subjected to two different mating treatments: multiple-drone instrumental insemination (IQ) and free-mating (FMQ).

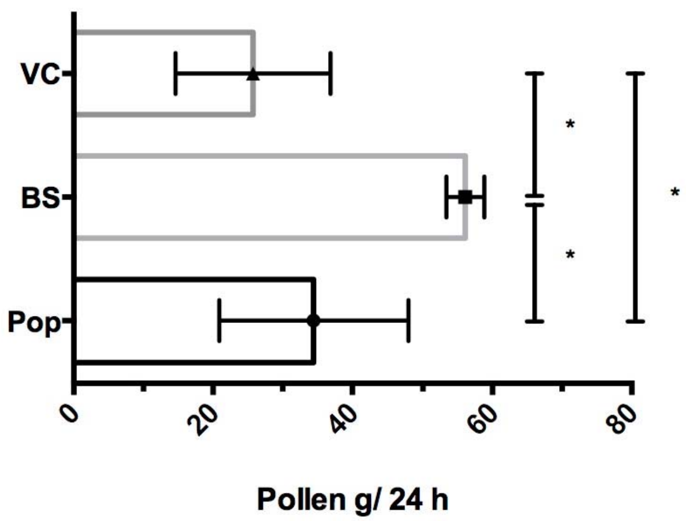
Multiple-drone instrumental insemination: Drones from Colony B were marked inside the hive, and 10 days later we collected semen from them. Ten queens, produced from Colony A, were instrumentally inseminated with an 8.0μL blend of semen collected from 12 different sibling drones from Colony B. After the instrumental insemination process, queens were introduced into queenless colonies and a queen-excluder screen was installed at the entrance of the hives to prevent the queens from attempting mating flights. After 10 days, the colonies were inspected and those starting to lay eggs were included in subsequent analyses.

Free-mating: Ten queens, produced from the same Colony A were introduced into queenless colonies with free access for mating flights. After 10 days of the queen introduction, the colonies that presented laid eggs continued for the next steps of the research. No other apiary was located within a 3km radius of the experimental apiary where the research was conducted. In this treatment group, we expected the queens to produce offspring with a wider genetic variability, since the drones that copulated with those queens were unlikely to be siblings and probably derived from different colonies. After 70 days, when the entire colony was replaced by the new queen's offspring, bee-pollen production was measured again. The mean bee-pollen weight collected was calculated for each colony.

A group of 20 randomly chosen colonies were introduced into the experimental apiary and maintained as a control group (C). Those colonies had the pollen production measures conducted at the same time as in the BS, IQ, and FM groups. These control groups were used to infer the effect of seasonality on the bee-pollen production. ANOVA was used to compare pollen production among different treatment groups. Tuckey's multiple comparison test was used as *post hoc* for pairwise comparison. The T test was used to compare the mean productive measures registered for the control colonies. The $\alpha$ error assumed for all statistical tests was 0.05. All statistical analyses were performed in SPSS (SPSS, 2013)

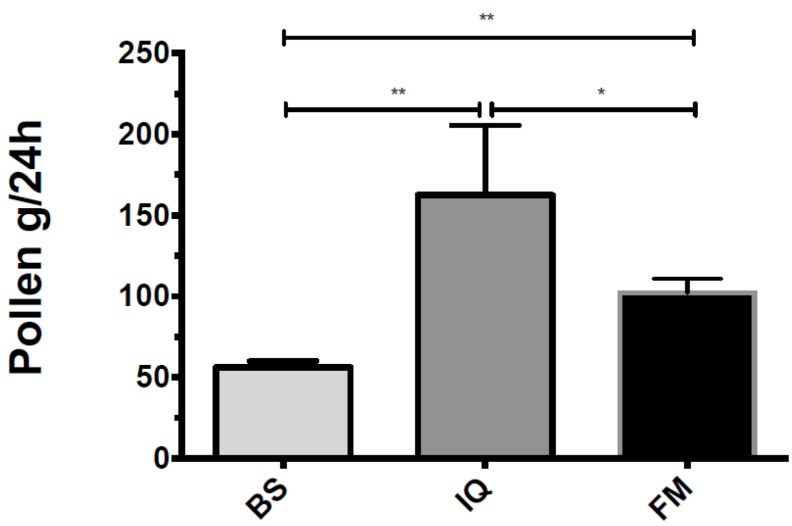
## RESULTS

Mean pollen collection of the initial 80 colonies ranged from 14.19 to 58.83g/day (mean= 34.32; SD= 13.50). The two colonies (BS) selected for the two treatments showed the highest pollen production: 58.83±23.11 and 53.37±31.20, for Colony A and B, respectively. The VC group produced 25.73±11.12g/day. Mean pollen collection between the BS group and the VC group was significantly different (Figure 1). We detected a significant increase in mean pollen production between the parental colonies (BS) and the colonies in the two treatment groups (BS *vs.* IQ: P<0.001; BS *vs.* FM: P<0.001). However, we did not detect a significant difference in pollen production among the different measures registered by control colonies (t= 1.799; P= 0.1056). The F1 generation of the IQ treatment produced 2.74 times more pollen than the parental generation (153.95±42.83). We also observed an increase in pollen production for the F1 generation of the FM treatment, 100.07±8.23, but the increase was only 1.78 times. The difference in pollen production between the IQ and FM treatments was also statistically significant (P= 0.003) (Figure 2).




*ANOVA: P< 0.001; *Tuckey's test: P< 0.001

Figure 1. The mean bee-pollen production (g/24 hours) registered in 80 colonies before selection (Pop), the highest pollen producer colonies (BS) and the colonies excluded of the breeding program (VC).




**ANOVA: P< 0.001; **Tuckey's test: P< 0.001; *Tuckey's test: P< 0.01

Figure 2. The mean pollen production (g/24 hours) observed in two different generations: Parental and F1 with two different methodologies of breeding (instrumental insemination – IQ, free-mating – FM).

## DISCUSSION

The results obtained suggest that instrumental insemination is an effective breeding technique for bee-pollen production. Even though we also observed an increase in pollen production with free-mating treatment, the selective approach using IQ queens allowed us to reach better outcomes in one-generation selection. These results are probably regardless from effects of seasonal changes in floral availability, as we did not observe a significant difference between registers of pollen production from control colonies. We already expected a large genetic effect from the pollen production trait given that the pollen-hoarding syndrome follows an additive inheritance system, driven by four quantitative trait loci (Hunt *et al*., 1995; Page *et al*., 2000; Rüppell *et al*., 2004; Rueppel, 2014). Despite the fact that our results reinforce the idea that the pollen-producing and pollen-hoarding traits can be correlated, we cannot assert that both phenotypes have the same heritability or even share the same genetic basis.

## CONCLUSION

Overall, we conclude that the IQ group presented significantly higher pollen production with a 53% increase in pollen production when compared to the FM group. Thus, the use of inseminated queens should be considered by beekeepers aiming to improve their bee-pollen production because it provides fast and, potentially, long-lasting results. It is important to emphasize that many questions regarding bee-pollen production remain to be answered. For example, whether these pollen-producing traits interact with those four QTLs associated with pollen-hoarding syndrome. However, this study presents a first step to investigate approaches that improve pollen-producing traits in honey bees for commercial use by beekeepers.

## ACKNOWLEDGEMENTS

The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial assistance.

## REFERENCES

ATTIA, Y.; ABD AL-HAMID, A.E.; IBRAHIM, M.S. *et al.* Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannan oligosaccharides continuously or intermittently. *Livest. Sci.*, v.164, p.87-95, 2014.

ATTIA, Y.A.; AL-HANOUN, A.; TAG EL-DIN, A.E. *et at.* Effect of bee pollen levels on productive, reproductive and blood traits of NZW rabbits. *J. Anim. Physiol. Anim. Nutr.*, v.95, p.294-303, 2011.

BUCK, A.C.; REES, R.W.; EBELING, L. Treatment of chronic prostatitis and prostatodynia with pollen extract. *Br. J. Urol.*, v.64, p.496-499, 1989.

BURGETT, M.; BURIKAM, I. Number of adult honey bees (Hymenoptera: Apidae) occupying a comb: a standard for estimating colony populations. *J. Econ. Entomol.*, v.78, p.1154-1156, 1985.

DHAR, N.B.; SHOSKES, D.A. New therapies in chronic prostatitis. *Curr. Urol. Rep.*, v.8, p.313-318, 2007.

EL-ASELY, A.M.; ABBASS, A.A.; AUSTIN, B. Honey bee pollen improves growth, immunity and protection of Nile tilapia (*Oreochromis niloticus*) against infection with *Aeromonas hydrophila*. *Fish Shellfish Immunol*., v.40, p.500-506, 2014.

GALLAI, N.; SALLESC, J.M.; SETTELED, J.; VAISSIÈREA, B.E. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.*, v.68, p.810-821, 2009.

HABIB, F.K.; ROSS, M.; BUCK, A.C. *et al*. In vitro evaluation of the pollen extract, cernitin T-60, in the regulation of prostate cell growth. *Br. J. Urol.*, v.66, p.393-397, 1990.

HANA, L.H.Y.; SHANB, S.; ZHANGA, X. *et al*. Down-regulation of prostate specific antigen in LNCaP cells by flavonoids from the pollen of *Brassica napus*. *Phytomedicine*, v.14, p.338-343, 2007.

HUNT, G.J.; PAGE Jr, R.E.; FONDRK, M.K.; DULLUM, C.J. Major quantitative trait loci affecting honey bee foraging behavior. *Genetics*, v.141, p.1537-1545, 1995.

KLEIN, A.M.; VAISSIÈRE, B.E.; CANE, J.H. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B Biol. Sci.*, v.274, p.303-313, 2007.

LAIDLAW Jr., H.H.; PAGE Jr., R.E. *Queen rearing and bee breeding*. Cheshire: Wicwas Press, 1997. 224p.

MAUGHAN, R.J.; EVANS, S.P. Effects of pollen extract upon adolescent swimmers. *Br. J. Sports Med.*, v.16, p.142-145, 1982.

PAGE, R.E.; FONDRK, M.K.; HUNT, G.J. *et al*. Genetic dissection of honeybee (*Apis mellifera* L.) foraging behavior. *J. Hered.*, v.91, p.474-479, 2000.

RINDERER, T.E.; HARRIS, J.W.; HUNT. G.J. *et al*. Varroa destructor in north America. *Apidologie*, v.41, p.409-424, 2010.

ROSENKRANZ, P.; AUMEIER, P.; ZIEGELMANN, B. Biology and control of *Varroa destructor*. *J. Invertebr. Pathol.*, v.103, p.96-119, 2010.

RUEPPELL, O. The architecture of the pollen hoarding syndrome in honey bees: implications for understanding social evolution, behavioral syndromes, and selective breeding. *Apidologie*, v.45, p.364-374, 2014.

RÜPPELL, O.; PANKIW, T.; NIELSON, D.I. *et al.* Pleiotropy, epistasis and new QTL: the genetic architecture of honey bee foraging behavior. *J. Hered.*, v.95, p.481-491, 2004.

SPSS statistics for windows. Version 22.0. Armonk: IBM Corp., 2013.

SZABO, T.I.; LEFKOVITCH, L.P. Fourth generation of closed population honey bee breeding relationship between morphological and colony traits. *Apidologie*, v.19, p.259-274, 1988.

TURNER, K.K.; NIELSEN, B.D.; O'CONNOR, C.I.; BURTON, J.L. Bee pollen product supplementation to horses in training seems to improve feed intake: a pilot study. *J. Anim. Physiol. Anim. Nutr.*, v.90, p.414-420, 2006.

Value-added products from beekeeping. rural infrastructure and agro-industries division, W0076, Rome: FAO, 1996. (Agricultural Services Bulletin, 124).

WANG, Y.K.; LIND, Y.; RAUWALD, H.W. *et al.* A phytosterol enriched refined extract of *Brassica campestris* L. pollen significantly improves benign prostatic hyperplasia (BPH) in a rat model as compared to the classical TCM pollen preparation qianlie kang pule'an tablets ruwei. *Phytomedicine*, v.22, p.145-152, 2015.

WILLIAMS I.H. The dependences of crop production within the European Union on pollination by honey bees. *Agric. Zool. Rev.*, v.6, p.229-257, 1994.

WU, Y.D.; LOU, Y.J. A steroid fraction of chloroform extract from bee pollen of *brassica campestris* induces apoptosis in human prostate cancer PC-3 cells. *Phytotherapy Res.*, v.21, p.1087-1091, 2007.

XU, J.; QIAN, W.Q.; SONG, J.D. A comparative study on different doses of cernilton for preventing the clinical progression of benign prostatic hyperplasia. *Zhonghua Nan Ke Xue*, v.14, p.533-537, 2008.

ZHANG, X.; HABIB, F.K.; ROSS, M. *et al.* Isolation and characterization of a cyclic hydroxamic acid from a pollen extract, which inhibits cancerous cell growth in vitro. *J. Med. Chem.*, v.38, p.735-738, 1995.