**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]

I.M. de Mattos, J. Souza, A.E.E. Soares

Universidade de São Paulo – Ribeirão Preto, SP

**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

**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...
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 Eucaliptus 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...
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).

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).
**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.

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**REFERENCES**


