Propolis production and its relation to wax production rate in *Apis mellifera* beehives

Produção de própolis e sua relação com a velocidade de produção de cera em colmeias de *Apis mellifera*

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Abstract

The aim of this work was to determine whether there is any relationship between wax production rate and propolis production. High (P1) and low (P2) propolis-producing hives were chosen in a selection process. Through controlled matings, four different groups of beehives were obtained: D1 hives, originating from the mating of P1 queens and P1 drones; D2 hives, originating from P1 queens and P2 drones; D3 hives, originating from P2 queens and P1 drones; and D4 hives, originating from P2 queens and P2 drones. After 110 days, one frame with wax foundation was put in the center of each hive and the areas that the honeybees drew out the comb were estimated. The propolis can be collected thirty days after placement of the collectors. Propolis is preferentially deposited on the sides of the collector that receive the greatest exposure to sun and wind, mainly in the winter. D1 beehives accumulated five times more propolis than did D2 and D3, and 34 times more than D4. D4 beehives drew out the combs more efficiently, when compared to the D1, D2 and D3 beehives.

Additional keywords: controlled matings, propolis collector, weather conditions, wax utilization.

Resumo

Os objetivos deste trabalho foram analisar a produção de própolis em colmeias descendentes de parentais com alta e baixa produção de própolis e se há relação entre velocidade de produção de cera e produção de própolis. Num processo de seleção, foram escolhidas as colmeias que mais (P1) e menos (P2) produziam própolis que, por sua vez, deram origem às colmeias D1: com rainhas descendentes de colmeias P1 acasaladas com zangões P1; D2: com rainhas descendentes de colmeias P2 acasaladas com zangões P1; D3: com rainhas descendentes de colmeias P1 acasaladas com zangões P2; e D4: com rainhas descendentes de colmeias P2 acasaladas com zangões P2. Estimou-se a área de alvéolos puxados num quadro com cera alveolada colocado nas caixas. A coleta da própolis pode ser feita a partir de trinta dias da colocação dos coletores de própolis. A própolis é preferencialmente depositada em laterais do coletor que recebem maior intensidade de raios solares e ventos, principalmente no inverno. As colmeias D1 acumularam cinco e 34 vezes mais própolis que as D2 e D3 e, D4, respectivamente. As colmeias D4 puxaram cera com maior eficiência, quando comparadas às colmeias D1, D2 e D3.

Palavras-chave adicionais: acasalamentos controlados, coletor de própolis, condições climáticas, utilização da cera.

Introduction

Through the harvest of resins, balsams, waxes, volatile oils and pollen, bees make propolis, a product that varies in the proportions of its components, a fact due to the biodiversity existing in the location where the hive is found, to seasonal variations and also to the genetic potential of the bees in harvesting these materials (MEYER, 1956; KERR et al., 1970; GHISALBERTI, 1979; MARLETTO & OLIVEIRA, 1981; FUNARI, 1985; CRANE, 1990; WOYKE, 1992; AZEVEDO, 1996). Thus, by installing the hives in regions with abundant amounts of plants that are sources of the constituents of propolis and through breeding studies, it is possible to obtain increased production of propolis, leading to greater earnings in apiculture (BREYER, 1995; MANRIQUE & SOARES, 2002; INOUE et al., 2007; PICKLER, 2009).

Propolis is utilized by the bees to cover holes, diminish the entrance, unite the frames, disinfect the honyecombs and mummify small animals that enter and die in the hives when the bees are unable to remove them (PARK et al., 1995; MARCUCCI, 1996). It is possible to collect propolis by scraping the top and sides of the hive; however, besides the production being small, the quality of propolis is reduced due to the presence of pieces of wood. The quantities of propolis produced can vary from 300 g a year, according to PROST-JEAN (1985), to 700 g hive^{-1} year^{-1}, through the implementation of stimulation techniques (BREYER, 1995).

One method for inducing the production of propolis is the placing a plastic screen between the top and the nest, which stimulates the covering of the screen with propolis. After two months the screen can be removed, rolled up, conditioned in a plastic bag and placed in a freezer. After 24 h, the propolis can be separated from the screen. Another method consists in removal of the side slats of the hives made specifically for the production of propolis. The bees cover the open space with propolis, and similarly, instead of removing the slat, a gradual opening of a window can be made, as the propolis is deposited. A simple method is the lifting the top with wedges of two to three centimeters in width, forming openings that are filled with propolis (COUTO & COUTO, 2006).

The wax is utilized for the construction of honeycombs and in the elaboration of propolis, representing up to 40.0% of the total (MARCUCCI, 1995). For commercialization, propolis should contain a maximum of 25% wax (COUTO & COUTO, 2006). BIENEFELD & PIRCHNER (1990), evaluating many colonies of *Apis mellifera* carnica, verified that there were negative correlations between direct and maternal genetic effect of -0.96 for the production of wax. The heritability estimative for queens and workers in the production of wax is 0.45 and 0.39, respectively (BIENEFELD & PIRCHNER, 1991).The objectives of this work were to determine 1) the production of propolis and its deposition on the four sides of the collectors placed in the hives submitted to a selection process and 2) the relation between propolis production and the rate of wax production in hives of *Apis mellifera*.

Material and methods

This work was carried out in the Sector of Apiculture of the School of Agricultural and Veterinary Sciences - UNESP, Campus de Jaboticabal. The city of Jaboticabal is situated in the northeast region of the state of São Paulo, with coordinates 21^{o}15'22'' S and 48^{o}18'68'' W, at an altitude of 595 m, with temperate sub-tropical climate and mean annual temperature of 21 ^{o}C. The mean rainfall is 1431mm. The region is characterized by sugar cane monoculture.

After following 36 hives, a selection was made of three good propolis producers and three poor producers, to comprise the group of parental hives. The selection was made according to the method described in the section on the production of propolis.

Young queens were produced from the parental hives by the method proposed by DOOLITTLE (1899). After being born, the queens were transferred to nuclei of four frames, with an orphaned hive for a period of 24 h. In these nuclei, the queens were trapped in cages for a period of 48 h. A screen was placed at the entrance to the hive impeding the queen from exiting. After 48 h, the queens were let loose and remained free inside the hives for another five days, a period in which they reached sexual maturity.

Next, the queens were inseminated, marked and transferred to their respective definitive hives, remaining trapped for two days in cages that made it possible for the queens to move about in the honeycomb and communicate with the workers, after which the queens were freed. After a period of seventy days, the workers were completely replaced by descendants of the matings and data collection was initiated (RINDERER, 2008).

The parental hives that showed the largest and smallest amounts of propolis were called P1 and P2, respectively. The controlled matings were carried out resulting in the following hives: D1, with queens derived from P1 hives mated with P1 drones; D2, with queens derived from P2 hives mated with P1 drones; D3, with queens derived from P1 hives mated with P2 drones; and D4, queens derived from P2 hives mated with P2 drones.

Artificial insemination was performed with the selected queens anesthetized with carbon dioxide, and fixed to a special apparatus, in which the queens were immobilized and their posterior part of the abdomen was kept open by means of hooks. In this manner, the sexual ducts of the females were exposed and semen from the selected drones was introduced (COBEY, 2007).

To obtain the drones from the parental hives, screens were placed on the entrance of the selected boxes one month before the date of insemination and one frame per hive was introduced with individual honeycombs for the queens to lay the eggs of the drones. The queens were imprisoned in these frames in appropriate cages, where they were released after ovoposition.

Four groups of descendants were evaluated, with five repetitions each, totaling 20 hives.

The production of propolis in the hives was determined by weighing the propolis deposited on the four sides of the collector which was placed between the nest and the top of the hives. The propolis collector had the same dimensions of length and width as those of the Langstroth hive and a height of two centimeters. At the ends, there were wood cubes and the sides, front and bottom were open, forming four windows, sites of propolis deposition. This type of collector facilitates the handling of the hive and has been by various beekeepers in Sao Paulo.

In the parental hives, the propolis collectors were left in the hives between July 28 and September 29 of 2003. The 36 hives that were included in the selection process were previously prepared by feeding with syrup (equal parts of water and sugar), making sure that all the hives had similar conditions with respect to the brood area, food (honey and pollen) and number of bees. This was achieved by biweekly visual inspection of all the frames of the hives and giving a score of one (weak hives) to five (strong hives), also during the selection process. The collectors were placed in the descendant hives on March 15 of 2004 and removed on April 19 of 2004.

After 120 days following inseminations, a frame with foundation was placed in the center of all the descendant hives. Every two days from the second to the tenth day after placement of the frame, the areas of the drawn combs were estimated utilizing the wood support which on the sides had outstretched wires. These wires made up the inside of the frame, creating squares with an area of 4 cm^2. The area of wax drawn was determined based on the number of squares counted (AL-TIKRITY, 1971).

The comparison of the four groups of descendants with regard to propolis production was carried out by analysis of variance, with a completely randomized design, using five repetitions. In the study of the rate of wax production, a completely randomized design was utilized in a parcel subdivided in time, with five repetitions. The comparisons of means were performed using Tukey's test and the data processed using SAS (1993).

Results and discussions

Of the 36 hives that were included in the selective process, seven were eliminated, because they showed low performance in the production of brood and food, besides not producing propolis. On average, the three hives chosen that produced high and lows amounts of propolis, accumulated 16.00 ± 7.70 g and 0.64 ± 0.54 g, respectively. The production of propolis did not show a normal distribution, since 16 hives (55.2% of the total) accumulated less than one gram of propolis in a period of two months (Figure 1).

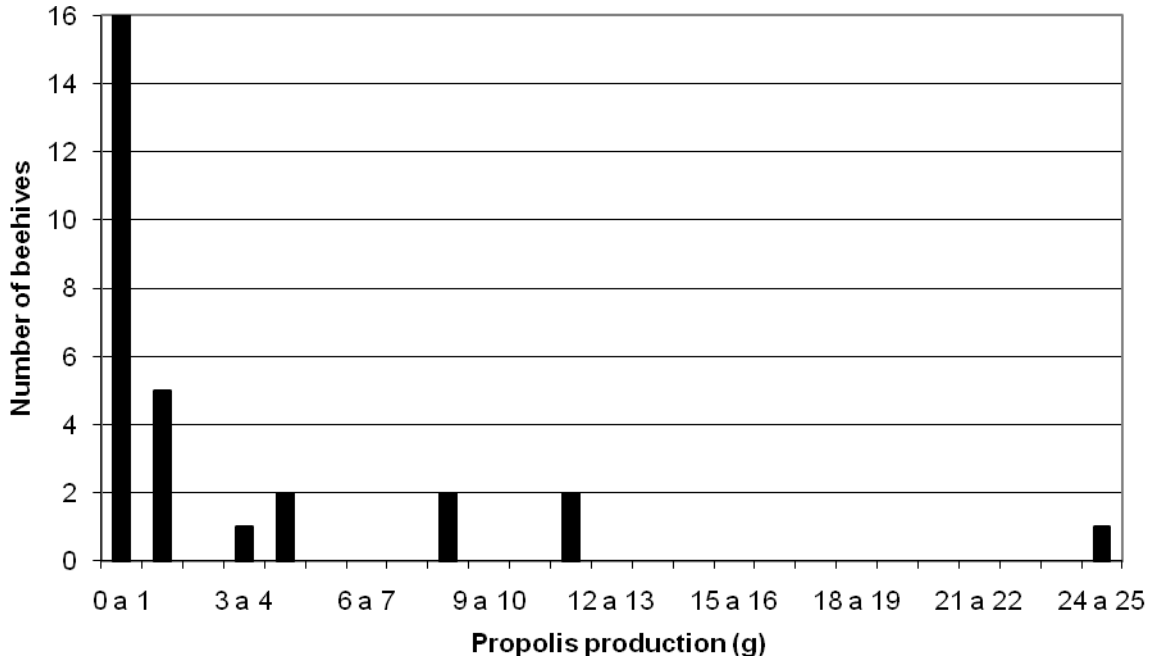

Figure 1 - Frequency of hives that were included in the selection process with respect to propolis production. *Frequência de colmeias que participaram do processo de seleção para produção de própolis.*

The small amounts of propolis obtained, even considering the most productive hives, are due to the fact that the region of Jaboticabal is characterized by sugar cane monoculture, possessing areas lacking plants that furnish raw materials for propolis.

The total propolis was obtained by summing the amounts from the four sides of the collectors. The hives, on average, deposited 47.0%, 26.9%, 18.2% and 7.9%, respectively on the left side, bottom, right side and front. In this winter period of low temperatures (July 28 to September 29, 2003), mainly during the morning, the bees tend to delay exiting the hive, which is stimulated by the presence of light (HEINRICH, 1979). The left side faced the east and was the first to receive the sun's rays, which explains why this side had the greatest deposition of propolis. The accumulation of propolis was greater in the bottom than in the front (entrance facing the sun), because in this period of the year, the sun travels a trajectory with an inclination toward the northern hemisphere, and thus there was stronger light in the back part of the collector. In this period of the year, there are also very strong winds compared to other periods.

Hives D1 produced, on average, 22.43 g of propolis which was statistically greater than that of hives D2, D3 and D4 which produced 4.28 g, 3.99 g and 0.65 g, respectively (Table 1). Despite the fact that there was no statistical difference among the hives that had at least one of their ancestors as a poor propolis producer, hives D2 and D3 accumulated respectively 5.58 and 5.14 times more propolis than did the hives with two ancestors selected for low propolis production.

Table 1 - F value, coefficient of variation (CV) and means with respective standard deviation (SD) obtained in the analysis of variance (data transformed into $Y^{1/4}$) and means with respective standard deviation between parentheses (data without transformation) of propolis production in each group of descendants. *Valor de F, coeficiente de variação (CV) e médias com respectivos desvios padrão (DP) obtidos na análise de variância (dados transformados em $Y^{1/4}$) e, médias com respectivos desvios padrão entre parênteses (dados sem transformação) de produção de própolis em cada grupo de descendentes.*

Statistics		Propolis production (g)		
F for descendant group		11.41 (P< 0.010)		
CV		31.4%		
Means ± SD	D1	[1] 2.12 ± 0.32	a	(22.43 ± 12.97)
	D2	1.33 ± 0.35	b	(4.28 ± 2.52)
	D3	1.29 ± 0.41	b	(3.99 ± 5.16)
	D4	0.58 ± 0.55	b	(0.65 ± 0.92)

[1]Means followed by same letters do not differ statistically (P>0.05), according to Tukey's test.

The collectors were left in the boxes for 35 days, 25 days less than in the hives used in the selection process. It was noticed that after the end of the first month there was no longer deposition of propolis in the collectors of parental and descendant hives. Evaluating propolis collection methods, INOUE et al. (2007), concluded that propolis production was not influenced by the tested methods ("intelligent collector, plastic screen and scraping), but seasonally had a marked effect. In this case the propolis collection occurred every month, for one year. KERR et al. (1970) also emphasize the importance of seasonality in the production of propolis. The seasonal patterns observed have been explained mainly by variations in temperature, sunshine, light intensity, relative humidity and precipitation. The less time the collector is left in the hive the better, so that the quality of the propolis is not affected by oxidation (ASIS, 1993). We suggest that new studies should be conducted to verify if the period of propolis collection can be defined for each season of the year.

The deposition of propolis occurred as follows: 40.1%, 39.7%, 17.1% and 3.1% on the right and left sides, bottom and front of the collectors, respectively. The amounts accumulated on the right and left sides were similar, different than what occurred in the selection process, when the left side showed 74.8% more propolis than the right side. In April and May, the higher temperatures (Table 2) and the milder winds compared to the climate during the selection period, provided conditions which contributed to the deposition of propolis being similar on both sides.

The sun followed a trajectory practically perpendicular to the boxes, without sun hitting the front and bottom of the box, indicating that the amounts of propolis in these locations should have been the same. This did not occur because of the slope of the land, as the boxes were inclined downward (side of entrance), making the entrance less exposed to the sun in relation to the bottom. It appears that the greater precipitation and relative humidity contributed to an increase in propolis production.

Table 2 - Means of climate data of the periods in which the propolis collectors were left in the parental and descendant hives, obtained from the Agroclimatology Station of the Department of Exact Sciences/UNESP, Jaboticabal Campus. *Médias dos dados climáticos dos períodos nos quais os coletores de própolis permaneceram nas colméias parentais e descendentes, obtidos na Estação Agroclimatológica do Departamento de Ciências Exatas/UNESP, Campus de Jaboticabal.*

Climate data	Parental hives Jul 28 to Sep 29, 2003	Descendant hives Mar 15 to Apr 19, 2004
Mean temperature (ºC)	21.1	23.0
Minimum temperature (ºC)	13.9	17.8
Maximum temperature (ºC)	29.7	31.1
Total precipitation (mm)	33.9	105.8
Relative humidity (%)	57.7	75.8
Wind speed (m/s)	1.95	1.77

D4 hives were statistically superior to the others, showing 192.0, 221.6, 280.8, 493.6 and 592.8 cm^2 of drawn wax, on the 2^{nd}, 4^{th} , 6^{th} , 8^{th} and 10^{th} days after the introduction of the frames with foundation (Table 3). Up to the first observation, D4 hives produced 21.82, 48.00 and 21.82 times more wax than did the hives D1, D2 and D3 (Tables 3 and Figure 2). Between the 2^{nd} and 6^{th} day, D4 hives drew 88.8 cm^2 of wax, which was only 3.7, 3.7 and 19.4% greater in relation to D1, D2 and D3, respectively. The performance of the hives D3 and D4 was similar between the 7th and 8th days, followed by D1 and D3. In the last two days, D4 hives showed the highest rate of wax production, with other treatments showing lower similar performance.

Table 3 - Means and respective standard deviation without parentheses [data transformed into log(x + + 1.5)] and means with respective standard deviation between parentheses (data without transformation) of areas of combs drawn out, at two-day intervals, up to the tenth day. *Médias e respectivos desvios padrão sem parênteses [dados transformados em log(x + 1,5)] e, médias com respectivos desvios padrão entre parênteses (dados sem transformação), das áreas de alvéolos puxados, em intervalos de dois dias, até o décimo dia.*

Descendant Groups	Day					General
	2^{nd}	4^{th}	6^{th}	8^{th}	10^{th}	
	Area (cm^2)					
D1	1.47 ± 1.49 (8.80 ± 3.97)	1.60 ± 1.66 (12.00 ± 17.9)	2.97 ± 2.51 (94.40 ± 121.41)	3.57 ± 3.99 (244.40 ±338.99)	3.81 ± 3.15 (314.40 ± 397.59)	2.68 ± 2.56 (134.80 ± 175.97) [2]b
D2	1.21± 1.11 (4.00 ± 5.66)	1.43 ± 1.40 (7.20 ± 9.96)	3.85 ± 1.94 (89.60 ± 53.93)	4.52 ± 2.31 (209.60 ± 130.86)	4.79 ± 2.46 (292.00 ± 174.68)	3.16 ± 1.84 (120.48 ± 75.02) b
D3	1.09 ± 1.53 (8.80 ± 19.68)	1.24 ± 1.87 (19.20 ± 42.93)	2.95 ± 2.41 (83.20 ± 121.17)	4.42 ± 2.28 (294.40 ± 357.46)	5.26 ± 1.62 (370.40 ± 363.67)	2.99 ± 1.94 (155.20 ± 180.98) b
D4	3.11 ± 2.86 (192.00 ± 295.82)	3.86 ± 2.45 (221.6 ± 312.10)	4.43 ± 2.47 (280.80 ± 319.87)	5.16 ± 2.69 (493.60 ±349.6)	5.78 ± 1.80 (592.80 ± 372.04)	4.47 ± 2.45 (356.16 ± 329.89) a
General	1.72 ± 1.75 (53.40 ± 81.28)	(2.03 ± 1.85) (65.00 ± 95.72)	3.55 ± 2.33 (137.00 ± 154.10)	4.42 ± 2.82 (310.50 ± 330.23)	4.91 ± 2.21 (392.40 ± 343.58)	3.33 ± 2.19 (191.66 ± 200.98)

[2] Means followed by same letters do not differ statistically (P>0.05), by Tukey's test.

In analyzing Figure 2, it is seen that the rate of wax production was greater in the descendants of hives that produced less propolis. Meanwhile, taking into account the basis of wax production, it is necessary to consider what will be its destiny in the hive, because it is known that besides being used in the construction of the honeycomb, wax is a component of propolis,

representing up to 40.0% of the total (MARCUCCI, 1995). The descendants of hives that were higher propolis producers directed a greater proportion of wax synthesized toward making this product, reducing the construction of honeycombs and vice-versa. MANRIQUE & SOARES (2002) verified a positive correlation between propolis and honey production (r=0,422). They observed that good producer propolis hives produced bigger amount of honey compared to the ones that did not produced propolis. However, PICKLER (2009), studying the relation of propolis production to others honey bees characteristics, observed that there are not correlation between propolis and honey production.

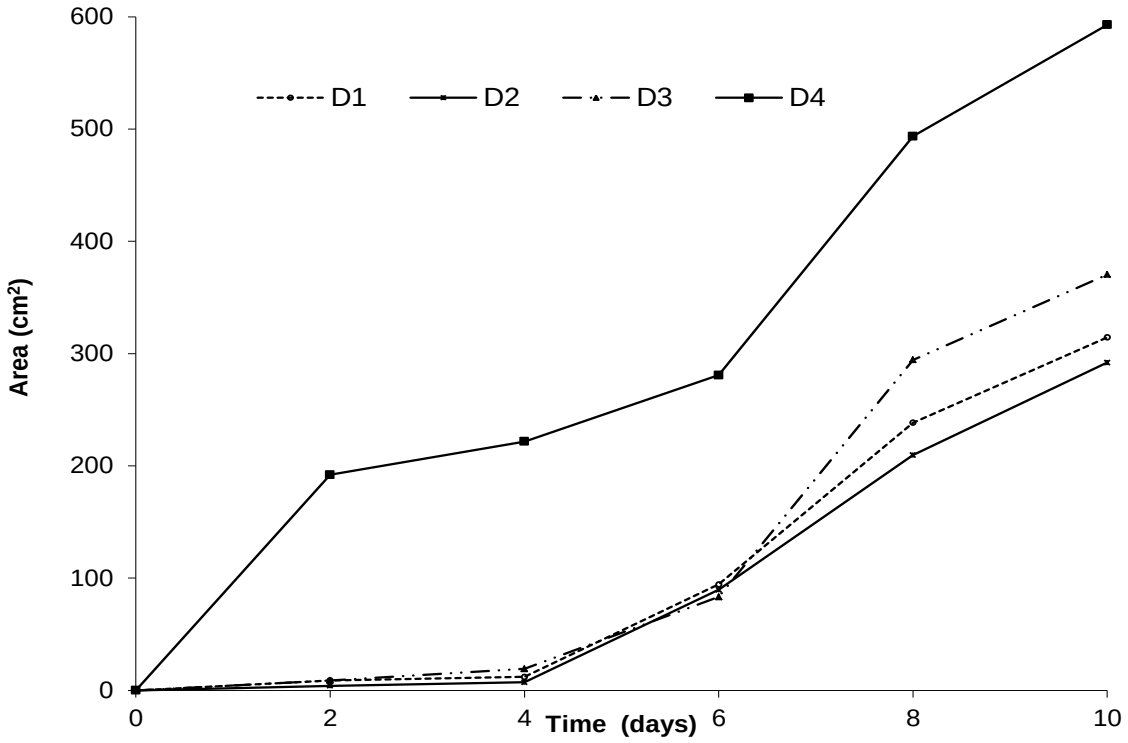

Figure 2 - Rate of wax production in hives D1, D2, D3 and D4, based on five observations carried out at two-day intervals. *Velocidade de produção de cera nas colméias D1, D2, D3 e D4, nas cinco observações realizadas, com intervalos de dois dias.*

Conclusions

Propolis is preferentially deposited on the sides of the box that receives the greatest amounts of sun and wind, mainly in the winter.

Hives derived from those with high rates of propolis production accumulated five and 34 times more propolis than did hives that had one or two ancestors from hives with low production, respectively.

Hives with low propolis production drew out wax with greater efficiency, when compared to hives that had at least one ancestor from hives with high propolis production. Considering the production of wax, it is necessary to think of its utilization, because hives that produce more propolis, direct wax to this product, reducing the honeycombs and vice-versa.

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