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Apitoxin harvest affects population development but not the hygienic behavior of African-derived honey bees

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Abstract

The biological properties of apitoxin have prompted its production for use in human and animal health applications. However, the apitoxin harvest triggers a defense reaction in honeybee colonies, which includes the release of alarm pheromones (isopentyl acetate and 2-heptanone), which cause stress and could cause behavioral changes that influence the routine activities of the colony. Considering the lack of data in the literature describing the effects of the prolonged harvesting of apitoxin, the present study conducted over a period of one year, aimed to investigate whether the apitoxin harvest influences population development and hygienic behavior of African-derived *Apis mellifera* (L.). We observed that apitoxin harvest affected the uncapped brood area of the colonies during the months of April, May, and June, and affected the capped brood area in July. The hygienic behavior of the colonies was not affected. Furthermore, we observed that during the study year, there was loss by abandonment of nine of the colonies subjected to apitoxin harvesting. We conclude that under the conditions of this study, the apitoxin harvest can negatively influence the development of the colony population during certain times of the year, without affecting the hygienic behavior of the colonies.

Introduction

Apitoxin is a product that bees can offer to humans, the word originating from the Latin *apis* (bee) and *toxikon* (venom.) This substance is produced by the acid glands of worker bees, and characterized as being clear, colorless, and highly soluble in water (Cruz-Landim & Abdalla, 2002).

Apitoxin toxicity is attributed to three proteic components: enzymes (phospholipase A2 and hyaluronidase), large peptides (melittin, apamin, and mast cell degranulating [MCD] peptide) and small molecules (peptides and biogenic amines), all substances that show pharmacological and allergenic activities. The allergenic factors are antigenic proteins such as phospholipases, hyaluronidases, lipases, and phosphatases, that are injected during the sting, and which initiate immune responses in hypersensitive individuals (Cardoso et al., 2009).

While the apitoxin can cause allergic and hypersensitivity reactions, small doses and fractions of its purified components have been used in the treatment of diseases such as arthritis, arthrosis, osteoarthritis, bursitis, tendonitis, multiple sclerosis, lupus, lower back pain, and sciatica (Ali, 2012). Apitoxin components have also been studied during the development of some cancer treatments (Orsolic, 2012) and for use against the human immunodeficiency virus (HIV) (Hood et al., 2013).

Thus, the biological properties of apitoxin have stimulated its commercial production (Leite & Rocha, 2005). For the utilization of apitoxin without the direct application of a sting, electric collectors are placed at the hive entrance, allowing the harvest of crude apitoxin without causing bee mortality (Benton et al., 1963; Leite & Rocha, 2005).

The bee venom harvest triggers a massive defense reaction in the colony, because when bees bump against the electric collector, they release a quantity of apitoxin and alarm pheromones (isopentyl acetate and 2-heptanone), which prompts other bees to do likewise. Although electric collectors

of apitoxin do not promote the bees' death, it is not known how much the prolonged harvest of apitoxin can influence colony activities. The assumption is that the prolonged harvest of apitoxin can promote acute and chronic stress, causing behavioral changes that interfere with the routine activities of the colonies. This can cause a decrease in the collection of resources by the colony, harming the development of broods and negatively affecting colony maintenance. It may also have a detrimental effect on the hygienic behavior of bees.

Considering that the harvesting of apitoxin is a growing part of beekeeping, and the lack of data describing its effects on colonies, the present study aims to investigate if harvesting apitoxin influences the population development and hygienic behavior of African-derived *Apis mellifera* (L.) over a period of one year.

Materials and Methods

The experiment was conducted at the Beekeeping Production Area of Lageado Experimental Farm, Faculty of Veterinary Medicine and Animal Science, UNESP, Botucatu, São Paulo, Brazil, 22°50′30.16″S; 48°25′41.90″W, with a humid subtropical (Cfa) climate and an average elevation of 623 m.

We used ten colonies of African-derived *A. mellifera* housed in wooden Langstroth hives, each containing six brood frames, three frames of food and one empty frame. During the trial period, hives were checked on a weekly basis, and when necessary each hive received sugar syrup (50% water; 50% sugar) using individual Boardman feeders. The colonies were free of diseases or parasites and each had a naturally mated queen, raised naturally by workers three months before the start of the experiment.

Before the start of the experiment, we performed defensive behavior tests on all of the colonies, according to the methodology described by Stort (1972) and Brandeburgo and Gonçalves (1990), in order to assess whether the colonies used showed uniform defensive behavior. The time to first sting and the number of stings left in a suede ball were recorded. The tests were performed in triplicate.

Subsequently, the colonies were randomly divided into two groups: control, without apitoxin harvest; and treatment, with a biweekly harvest of apitoxin. The apitoxin harvest was always conducted in the morning, beginning at 09:00 AM and continuing for an hour, by using an electric collector. In experiments carried out by our group, it was found that this timing of the harvests showed the best rates of toxin production, and exerted less stress on the colonies (as estimated by the expression of genes related to stress) (data in press). At the end of each apitoxin harvest, the collectors were removed and sent to the laboratory. The glasses containing the poison were kept at room temperature and shielded from exposure to light, to evaporation of the volatile phase. Then, the apitoxin was scraped out with a stainless steel spatula, weighed, stored in an Eppendorf tube, and kept in a freezer at -10 °C. After each harvest, the glasses were washed in running water and sanitized with 70° GL alcohol.

The population development of colonies was evaluated according to methodology adapted from Al-Tikrity et al. (1971). For this purpose, two frames containing posture and/or uncapped brood areas were removed from the colonies, labeled, and placed in a holder, with a grid comprised of squares of 2 cm × 2 cm. The number of squares with posture and/or uncapped brood areas was counted, and the frames were then returned to the hives. Fifteen days later, the labeled frames had their brood area reanalyzed (to verify the quantity of capped brood present). This process was repeated every two weeks throughout the trial period.

The hygienic behavior of all colonies (control and treatment) was analyzed by the method of drilling of capped brood, in accordance with the method described by Garcia et al. (2013). In treatment colonies, after apitoxin harvest, a frame with capped brood areas of each colony was selected, and two different areas containing approximately 100 cells (area A and B) were marked. One area of capped brood (area A) was drilled with an entomological pin, and the frame was returned to the hive and re-evaluated after 24 h. For analysis, the total number of capped brood subjected to drilling was counted (area A), and the total number of empty cells was subtracted from the number of cells drilled, to obtaining the number of pupae removed by hygienic workers. In area B, we calculated the factor of correction Z described by Moretto (as cited in Abdel-Rahman, 2014). Z is the natural taxa of removed pupae in a corresponding area, which is subtracted from the value of removed pupae in the area A. The result was considered if the value of the factor correction (area B) was equal to or less than 10%.

The Z value was calculated using the formula: $Z = (Y \times 100)/A$, where A = the number of pupae in the area A and Y = the number of empty cells where the pupae were removed naturally. The value of Y is given by $Y = C - B$; where C = number of empty cells from control after the frame has been subjected to a hygienic behavior test and B = the number of empty cells in the control, before the frame has been subjected to a hygienic behavior test.

The hygienic behavior rate (HBR) of each colony was calculated via the formula $HBR = (CV1 - CV \times 100 - Z)/CO$; where CV1 = the number of empty cells 24 h after drilling, CV = the number of empty cells before drilling, CO = the number of cells capped before drilling, and Z = the correction factor obtained in the control area.

Data analysis was performed by ANOVA, followed by the unpaired Student's *t*-test for comparing means, and considered significant when $P < 0.05$ (Zar, 2010).

Results

The time to first sting was 3.12 ± 2.10 s for the swarms of the control group, and 3.12 ± 3.04 s for the treatment group. The number of stings left in the suede ball in the control

colonies was 32.87 ± 18.81 and 36.12 ± 20.15 in the treatment colonies. These results for the defensiveness parameters did not differ significantly between the control and treatment colonies (P > 0.05; Student's *t*-test).

The population development of the colonies demonstrated significant reduction in uncapped brood areas during the months of April, May, and June in colonies where apitoxin was harvested. Additionally, the areas of capped brood were reduced during July (Table 1).

The hygienic behavior test did not show a significant difference between colonies with or without the harvest of apitoxin (P > 0.05; Student's *t*-test) (Table 2).

Table 1. Means and standard deviations of the uncapped and capped brood areas (cm^2) of African-derived *Apis mellifera* (L.) colonies without (Control) and with the harvest of apitoxin (Treatment), between April 2013 to March 2014.

Months	Uncapped brood		Capped brood	
	Control	Treatment	Control	Treatment
April	60.93 ± 12.45	34.22 ± 9.31[1]	165.91 ± 30.72	147.54 ± 17.93
May	122.75 ± 19.73	78.22 ± 18.63[1]	131.62 ± 44.24	87.61 ± 15.05
June	70.94 ± 18.45	53.36 ± 12.72[1]	115.12 ± 65.14	32.73 ± 14.04
July	39.84 ± 19.63	30.82 ± 8.45	108.70 ± 20.56	23.74 ± 19.30[1]
August	27.22 ± 10.15	20.33 ± 4.06	25.74 ± 8.43	20.32 ± 4.07
September	22.35 ± 9.54	14.13 ± 5.05	120.83 ± 71.55	107.34 ± 56.55
October	52.35 ± 23.54	25.93 ± 15.62	97.52 ± 71.85	158.26 ± 34.56
November	36.23 ± 9.45	32.76 ± 15.63	119.13 ± 17.21	173.42 ± 41.05
December	63.44 ± 17.32	42.05 ± 13.32	101.94 ± 65.26	126.56 ± 52.24
January	40.24 ± 21.42	77.94 ± 40.23	128.05 ± 58.07	221.40 ± 40.22
February	43.34 ± 19.25	65.15 ± 12.82	148.51 ± 62.35	151.71 ± 37.43
March	50.86 ± 7.83	75.91 ± 32.96	134.05 ± 42.02	146.46 ± 15.36

[1]Significantly decreased compared with control (P < 0.05 Student's *t*-test). n = 5 colonies.

Table 2. Means and standard deviations of the hygienic behavior test (%) of African-derived *Apis mellifera* (L.) colonies without (Control) and with the harvest of apitoxin (Treatment), between April 2013 to March 2014.

Months	Control	Treatment
April	84.63 ± 13.86	79.95 ± 6.85
May	81.26 ± 14.74	71.95 ± 17.22
June	83.65 ± 7.13	80.76 ± 10.97
July	88.96 ± 11.77	87.82 ± 11.56
August	79.23 ± 9.36	75.54 ± 11.47
September	91.57 ± 5.12	84.34 ± 8.56
October	90.25 ± 6.37	74.35 ± 14.86
November	82.00 ± 12.40	92.64 ± 6.76
December	72.10 ± 30.20	91.13 ± 1.55
January	80.22 ± 3.86	69.90 ± 22.14
February	84.63 ± 4.86	73.64 ± 11.44
March	86.12 ± 6.14	71.33 ± 16.84

No significant differences were observed in hygienic behavior between treatment and control colonies. Student's *t*-test (P > 0.05). n = 5 colonies.

Discussion

To ensure a similar degree of defensiveness in colonies used in our study, we evaluated the defensive behavior of all colonies before the start of the experiment. We noted that the colonies showed similar patterns; however, this differed from reports in the literature (Nascimento et al., 2008). These results could be ascribed to environmental factors (temperature, humidity, and atmospheric pressure) and/or genetic factors (Garcia et al., 2013). Therefore, it is arguable that the similarities in the defensive behaviors seen in the colonies used here allow this study to avoid possible genetic effects that could influence our results.

It was verified that the harvest of apitoxin decreases the area of uncapped brood during the months of April to June, and decreases the area of capped brood in the month of July, when compared with the controls. This decrease in brood area could be associated with the release of alarm pheromones that may change honeybee behavior, mainly by interfering with the flux of information transmitted by other pheromones in the colonies (Kastberger et al., 2008). Thus, the collecting of apitoxin could interfere with brood care, the queen's oviposition of worker eggs, and with resources collection by worker bees in some periods.

One important observation made by this study is the difficulty of maintaining colonies that are submitted to the harvest of apitoxin. Between the months of August and January, nine colonies from which apitoxin was collected abandoned their hives, and had to be replaced with new colonies showing similar defensive behaviors. This abandonment behavior is common in African-derived honey bees and is characterized by all in the colony bees abandoning the hive (Freitas et al., 2007). It is usually observed during stress conditions, during periods of climatic change or when resources are scarce, which are factors that can threaten the survival of the colony. It is suggested that the high rate of abandonment observed in our study could be caused by the collection of apitoxin, as the abandonment behavior was not observed in the control hives.

We noted that the harvest of apitoxin did not affect the hygienic behavior of the colonies, suggesting that the release of alarm pheromones does not interfere with this activity. The removal of sick or dead bees from the hive is associated with the social immunity of bees, as it prevents the spread of pathogens and increases the bees' resistance to diseases (Wilson-Rich et al., 2009). African-derived bees exhibit more intense hygienic behavior than European bees, which helps fight parasites and pathogens that cause disease in bees (Aumeier et al., 2000; Guerra, 2000).

On the basis of our results, future studies must consider the effects of apitoxin harvest at different time intervals, in order to develop further sustainable methods of apitoxin harvest that may be carried out throughout the year without affecting the development of hives and to avoid abandonment of colonies.

Thus, we conclude that apitoxin harvest negatively affects population development during certain times of the year, although it does not affect the hygienic behavior of colonies.

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