

Original Article

Behavior and mortality of *Apis mellifera* workers feeding on soft drinks in an urban environment

Comportamento e mortalidade de operárias de *Apis mellifera* alimentando-se de refrigerantes em ambiente urbano

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Abstract

Honeybees (*Apis mellifera* L.) (Hymenoptera: Apidae) coexists with humans in urban habitats foraging, mainly, sources containing carbohydrates. The behavior and risks for bees using soft drinks in their diet are poorly studied. The time spent by bee workers visiting soft drink leftovers in plastic cups and its death were evaluated. Bee workers spent more time visiting soft drinks in plastic cups (200 mL) than flowers of *Schinus* sp. (Anacardiaceae), *Anadenanthera* sp. (Fabaceae), *Vernonia* sp. (Asteraceae), *Citrus sinensis* L. (Rutaceae) and *Cissus rhombifolia* Vahl (Vitaceae), the five most visited honey plants in the field. Drowning mortality and the bee preference varied with the amount of soft drinks leftovers in cups. We present the first study of behavior and drowning mortality of *A. mellifera* feeding on leftover soft drink in plastic cups in urban areas, in the Brazilian Cerrado biome. This study may help to understanding the adaptive mechanisms of honeybees to explore habitats dominated by humans and unusual diets.

Keywords: Apidae, behavior, carbohydrates, foraging, honeybees.

Resumo

Apis mellifera L. (Hymenoptera: Apidae) coexiste em habitats urbanos com humanos forrageando, principalmente, fontes contendo carboidratos. O comportamento de forrageamento e os riscos para abelhas que buscam refrigerantes em sua dieta são pouco estudados. O tempo gasto por operárias para visitar restos de refrigerante em copos plásticos e sua morte por afogamento foram estudados. As operárias passaram mais tempo visitando refrigerantes em copos plásticos (200 ml) do que flores de *Schinus* sp. (Anacardiaceae), *Anadenanthera* sp. (Fabaceae), *Vernonia* sp. (Asteraceae), *Citrus sinensis* L. (Rutaceae) e *Cissus rhombifolia* Vahl (Vitaceae), que são as cinco plantas melíferas mais visitadas no campo. A mortalidade por afogamento e a preferência das abelhas variaram com os níveis de restos de refrigerante em copos plásticos. Apresentamos aqui o primeiro relato de *Apis mellifera* em áreas urbanas, no bioma Cerrado brasileiro, alimentando-se com restos de refrigerante em copos plásticos. Este estudo pode ajudar a entender os mecanismos adaptativos de abelhas selvagens para explorar habitats dominados por humanos e dietas incomuns.

Palavras-chave: Apidae, comportamento, carboidratos, forrageamento, abelhas.

1. Introduction

Honeybees have been associated with urban areas, although with a recognized ecological function as pollinators of angiosperm plants, in native and cultivated environments (Nascimento et al., 2020). The bees' presence in urban areas increases their likelihood of exploiting human food sources (Penick et al., 2016). Foraging bees consume part of the sugary sources found in flowers (in

the form of nectar and pollen) for energy and return to the hive (Woyciechowski, 2007), but they may also forage on sweetish exudations, originating from glands of vegetative parts of plants (Extrafloral nectaries) or processed by-products (Brod Schneider and Crailsheim, 2010).

Soft drinks contain large amounts of dyes, artificial preservatives, flavorings, and sugars, but with low

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nutritional content (Gardener et al., 2018). While honeybees can use this unusual food source, they spent less time contacting their proboscis on filter papers saturated with diet soft drinks than on conventional, higher-sugar soft drinks (Abramson et al., 1997). This suggests bees can detect sugar in these drinks and develop a preference based on that cue.

In every colony there are bees that search for water and bees that search for nectar (Robinson et al., 1984). Liquid sources of resources foraged by bees, such as water, prove the high social level of these insects. Water collection is regulated according to the current demand in the colony. The regulation of water collection is similar to that of nectar. The discharge rate of water foragers indicates the demand of the colonies for water (Woyciechowski, 2007; Kovac et al., 2010; Stabentheiner and Kovac, 2016; Kovac et al., 2018). Water is not an energy source and it increases the risks for foraging bees when returning to the colony. Bees with water in their crop have no metabolic fuel reserves, so they face a greater risk of not returning to the nest than nectar-foraging bees. Bees that fly longer distances to water sources fuel their foraging flights with more sucrose. Therefore, they prefer to collect water in the vicinity of the hive. For this reason, beekeepers maintain a clean water source near their hives (Kovac et al., 2010). On the other hand, the benefits of water for the hive, such as thermoregulation, to dilute stored honey, and for the consumption of nurse bees to produce jelly for feeding the larval brood (Kühnholz and Seeley, 1997; Ostwald et al., 2016), encourage bee workers to seek it even with risks (Stabentheiner and Kovac, 2016).

Soft drinks represent a hybrid source of dietary resources, with energy levels (kcal) and carbohydrates, such as found in pollen (source of protein, which generates energy) (Vanderplanck et al., 2014; Vaudo et al., 2015) and nectar (source of sugar) (Vaudo et al., 2015; Baude et al., 2016), respectively.

Bees forage soft drinks, but behavioral and risks associated with this diet should be studied. Edrees et al. (2019a, b) demonstrated that bees fed Pepsi were strongly influenced by deficiencies in essential nutrients. They observed a decrease in the number of bees and negative impact on the quality of honey and developing workers because of the lack of essential nutrients in Pepsi, causing high rates of mortality. Additionally, bees are at risk of drowning in soda depending on the challenge of accessing the beverage in a cup, can, or bottle. The objective of this study was to evaluate the time spent by *Apis mellifera* (Hymenoptera: Apidae) workers to visit soft drinks in disposable plastic cups compared to flowers from regional plants and the preference and risk of death due to different levels of soft drinks leftovers, commonly found after human consumption, in these cups.

The bioassays, were carried out to answer two questions: (1) would the bee time spent to visit soft drinks leftovers be shorter than in the main honey plants in the region? (2) whether bees preferred plastic cups

with different soft drinks leftovers levels and what was the risk of their death from drowning?

2. Material and Methods

The study was carried out due to the observation of bees in a school canteen at the Goiano Federal Institute, Urutaí campus, Goiás state, Brazil (latitude 17°29'10 "S, latitude 48°12'38" and 697 m altitude). A total of 210 students attended the canteen area daily, during the experimental period, from Monday to Friday, with an average consumption of 83.32 doses of soft drinks, in plastic cups (200 mL), in the morning (9:00 AM) and 186.65 in the afternoon (15:00 PM). The soft drink used contained caffeine, carbonated water, dyes, flavorings and sugar (Coke).

The time spent (in seconds) for the bees to access the soft drinks leftovers was recorded with a digital timer, from the moment the worker bee landed on the edge of the plastic cup (a), directed its mouth parts and accessed the liquid (b) and flew away from the source (c). The time spent by bees, which landed on the edge of the cups with soft drinks, but did not access the liquid, was discarded.

Foraging, in the five most visited honey plants in the region, was observed between 7:00 A.M. and 11:00 A.M (The flowers of these plants are open during this period). These plants were chosen according to information by beekeepers, in decreasing order of importance: *Schinus* sp. (Anacardiaceae), *Anadenanthera* sp. (Fabaceae), *Vernonia* sp. (Asteraceae), *Cissus rhombifolia* Vahl (Vitaceae) and *Citrus sinensis* L. (Rutaceae). All plants were available locally. Forty replications of the bee foraging time were carried out in the field by plant species. The time spent by the bee to access the flower included the behavior of landing (a), directing its oral parts in the reproductive structures (b) and leaving (c) the flower.

The amount of soft drinks left by canteen consumers in the cups was defined on a scale with levels 1, 2 and 3 (Figures 1 and 2) with >75%, up to 75% and <25% of the volume (200 mL) of the plastic cup filled with soft drinks, respectively. Forty replications were performed in a completely randomized design. The experimental unit consisted of a bee, a plastic cup and one of the three leftover levels established. This experimental design allowed to defining the preference (%) of bees according to the soft drink's leftovers and their mortality (%). The cups were observed in the canteen area to prevent the liquid from evaporating if they were taken to the field.

The data of the time spent to visits and mortality of drowning bees was submitted to ANOVA, with its means compared using the Duncan multiple amplitude test ($P=0.05$). The data on preference for visiting bees were analyzed using the non-parametric test of two samples, Wilcoxon-Mann-Whitney test ($P=0.05$). The SAEG® software, version 9.1 (UFV, MG, Brazil) was used to perform ANOVA and compare the means. The figures were created using SigmaPlot® software version 11.0 (Systat Software Inc, San Jose, CA, USA).

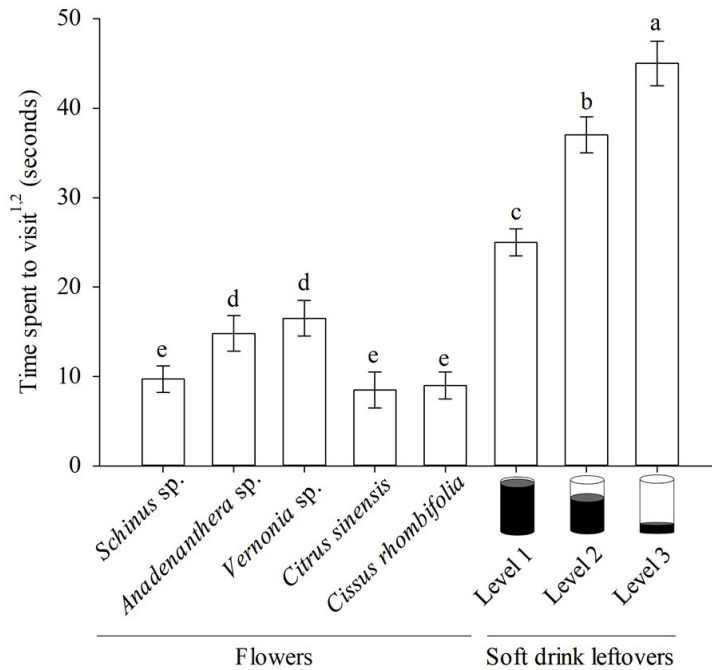


Figure 1. Time spent to visit (seconds) (Mean $\pm$ SE1) by *Apis mellifera* (Hymenoptera: Apidae) workers with two diets: flowers in the field or soft drinks in plastic cups at the college canteen. 1Means followed by the same letter do not differ by Duncan's multiple range test ($P > 0.05$). 2Period spent by the bees on flowers in the field was recorded through the following steps: (a) landed on the flower, (b) pulled its mouthparts at the reproductive structures of the flower and (c) leaving the flower. The period spent by the bees to forage on soft drinks in the cups was recorded from the moment that (a) the worker bee landed at the soft drinks cup edge, (b) pulled its mouthparts in the liquid and (c) left the source.

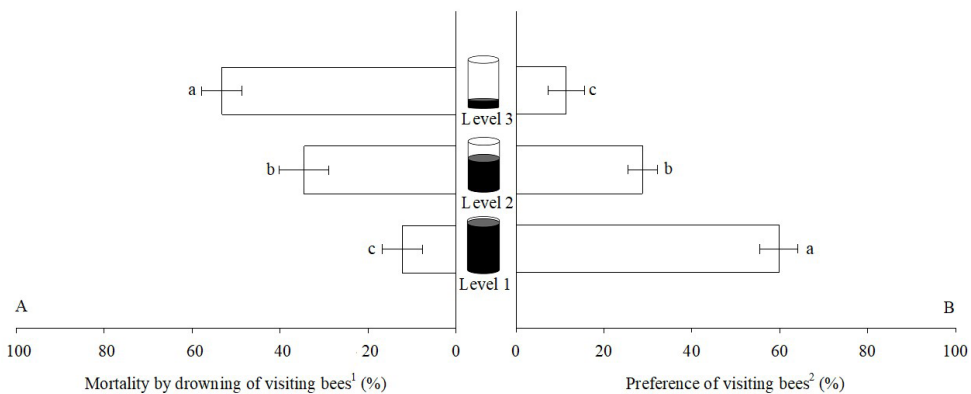


Figure 2. *Apis mellifera* (Hymenoptera: Apidae) mortality (%) by drowning (Mean $\pm$ SE1) and preference of visiting bees (Mean $\pm$ SE2) (%) to forage soft drinks in plastic cups at the college canteen. Means followed by the same letter do not differ by Duncan's multiple range1 ($P > 0.05$) or by the Wilcoxon-Mann-Whitney2 test ($P > 0.05$).

3. Results

The time spent by the bees foraging soft drinks was longer than in the honey plants ($F = 22.12$, $P = 0.02$) (Figure 1). Bees spent more time to forage *Anadenanthera* sp. and *Vernonia* sp. flowers than those of *Schinus* sp., *Citrus sinensis* and *Cissus rhombifolia* throughout the trial (Figure 1). The time spent by bees was shorter at level 1 (25.38 ± 2.67 seconds) than at levels 2 ($35.45 \pm$

3.76 seconds) and 3 (45.57 ± 3.78 seconds) considering only the soft drinks leftover left on cups (Figure 1).

The death of bees by drowning was greater in soft drink cups with level 3 than in those with 2 and 1 ($F = 31.25$, $P = 0.00$) with values of 53.22 ; 34.56 and 12.22% , respectively (Figure 2). Bees preferred soft drinks cups with level 1 over 2 and 3, with values of 59.81 ; 28.76 and 11.43% , respectively ($F = 8.96$, $P = 0.001$) (Figure 2).

4. Discussion

The greater time spent by the bees to visit soft drinks leftovers in the plastic cups, than in the flowers of the evaluated plants, is contradictory due to the easy access of the first ones. Even with its edge serving to land them, but this did not reduce the time spent for visitation. This indicates that the rate of ingestion (in quantity) must be more important for bees that seek liquid resources such as water. Soft drinks are nutritionally weak but also contain water, so collector bees may also be seeking a source of water in the soft drink. Although the nutrients in soft drink are not adequate, the bee needs to fly shorter distances to find sugar, due to the greater availability of the drink in plastic cups compared to flowers. Bees that collect nectar and pollen fly longer distances and expend more energy (Stabentheiner and Kovac, 2016). Therefore, the greater time spent to access the leftover soft drinks may be due to the process of liquid accumulation at the intestine level, facilitated by its high storage capacity with muscles allowing its expansion (crop). Honey bees have a crop in the anterior intestine (honey vesicle) where they store nectar and other liquids (Sammataro and Cicero, 2010).

The longest visitation time in *Anadenanthera* sp. and *Vernonia* sp. was, probably, due to these plants being the only ones with dense inflorescences, with stamens and stylets positioned in the center of the flowers, serving as a physical shield for the floral nectar. Suggesting that bees spend more time on flowers where nectar is harder to access. In these cases, bees tend to visit fewer flowers by inflorescence to optimize visitation time with lower energy expenditure (Akter et al., 2017).

The shade environment of the canteen may also have influenced the longer time for the bees to ingest soft drinks for energy replacement. The canteen environment is shaded throughout the day, has a roof, and the temperature is lower than in the external environment where there is sunlight. The use of solar heat to maintain thoracic temperature, where the muscles for flight are located, allows greater energy savings and, therefore, efficiency in the collection of floral resources (Kovac et al., 2010). Shaded environments (in our case, the school canteen) increase energy expenditure by bees to keep their muscles at a high enough temperature (between 35–41 °C) for immediate takeoff (Schmaranzer, 2000). Bees have to deal with enormous heat loss and therefore high thermoregulation costs, these high costs require energy optimization (Stabentheiner and Kovac, 2016). The shorter time spent to access soft drinks leftovers in level 1 (> 75% of the cups volume) may have been due to the association between their edges as a landing support and the appropriate length of the bee proboscis. The length of the oral structures determines the habit of foraging bees (Cariveau et al., 2016).

The higher mortality of bees by drowning in soft drinks cups with level 2 and, mainly, level 3 indicates that they were attracted or forced to explore the resource in these conditions, with a slippery surface, increasing drowning. The lower risk of drowning in level 1 cups determined the greater preference, aided by the effect of the edge and the

length of the bee mouth parts, two advantages combined previously discussed. In addition, the attraction for the soft drink, with flavorings in its composition, may have encouraged bees to explore the levels even at greatest risk using olfactory cues to identify foods (Ruedenauer et al., 2018). The ecological reasons that forced the bees to take risks to obtain the resource with greater chances of drowning were not identified. But they may be due to the lesser quantity of flowers in the native plant species and, therefore, less natural food supply in the summer of the Brazilian Cerrado biome, when this research was conducted. The scarcity of floral resources in the field (Abou-Shaara, 2014) and/or its low quality with the presence of pesticides applied for plant protection (Rodney and Purdy, 2020), for example, can affect the foraging and food choice by bees. Suggesting that if the environment near the colony has availability of nectar and pollen in plants (natural source) the bees will probably be less attracted to the soft drinks. Bees probably seek energy sources as close as possible to their colonies. The soft drinks leftovers represented by level 1 favored access to this resource. New studies with soft drinks should be carried out in places with many flowers (high availability of nectar and pollen) to identify the insect's preference.

This is the first report of *Apis mellifera* in urban areas in the Brazilian Cerrado biome (the second largest in Brazil) foraging on unusual diets, soft drinks. The use of pesticides in agriculture, deforestation of native forests and urbanization have been reducing bee biodiversity (Boscolo et al., 2017; Remmers and Frantzeskaki, 2024). Bees are increasingly adapting to urban environments by foraging unusual food sources (Penick et al., 2016). In cities, the high availability of sugar-rich foods has attracted some species of these insects to urban areas. Plant diversity positively influences bee species richness (Remmers and Frantzeskaki, 2024). For making cities bee friendly and bee inclusive, it is recommended to maintain natural areas, connect natural areas to urban ecosystems, encourage floral abundance and diversity and increase the size of urban green areas overall (Remmers and Frantzeskaki, 2024).

The inclusion of soft drinks in the diets of wild bees indicates a certain adaptation to the risks of this activity by visiting leftovers levels in the cups that led to lower bee mortality. Hybrid characteristics, as a source of sugar and liquid nature, of soft drinks can contribute to evaluate hypotheses to understanding the ecological services performed by foraging bees and the quality of this diet for other members of the hive.

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