REPELLENCE AND ATTRACTION OF *APIS MELLIFERA* FORAGERS BY NECTAR ALKALOIDS*

Z. Hroncová[1], J. Havlík[1], L. Stanková[1], S. Hájková[2], D. Titěra[2,3], V. Rada[1]

[1]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Prague, Czech Republic
[2]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Zoology and Fisheries, Prague, Czech Republic
[3]Bee Research Institute at Dol, Libčice nad Vltavou, Czech Republic

Plant secondary metabolites present naturally in nectar, such as alkaloids, may change the behavioural responses of floral visitors and affect pollination. Some studies have shown that nectar containing low concentrations of these secondary metabolites is preferred by honey bee foragers over pure nectar. However, it remains unclear whether this is caused by dependence or addictive behaviour, a simple taste preference, or by other conditions such as self-medication. In our choice experiment, free-flying bees were presented with artificial flowers holding 20% sucrose containing 0.5–50 µg ml^{-1} of one of the naturally occurring nectar alkaloids – caffeine, nicotine, senecionine, and gelsemine. Nectar uptake was determined by weighing each flower and comparing the weight to that of the control flower. Our experimental design minimized memorizing and marking; despite this, caffeine was significantly preferred at concentrations 0.5–2 µg ml^{-1} over control nectar; this preference was not observed for other alkaloids. All of the compounds tested were repellent at concentrations above 5 µg ml^{-1}. We confirmed previous reports that bees exhibit a preference for caffeine, and hypothesize that this is not due only to addictive behaviour but is at least partially mediated by taste preference. We observed no significant preference for nicotine or any other alkaloid.

nectar preference, caffeine, nicotine, senecionine, gelsemine

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INTRODUCTION

A characteristic feature of higher plants is their capacity to synthesize a variety of organic molecules known as secondary metabolites, which can protect them against a wide variety of pests (Wink, 1988). Several adaptive hypotheses have been proposed to explain the ecological and evolutionary roles of secondary metabolite alkaloids in nectar. They may deter nectar robbers (Johnson et al., 2006), prevent microbial degradation of nectar (Herrera et al., 2009), enhance cross-pollination by encouraging pollinators to move more quickly among flowers (Adler, 2000; Kessler, Baldwin, 2007), permit insect self-medication (Gherman et al., 2014; Baracchi et al., 2015), or even enhance connections between plants and certain insect species by eliciting addictive behaviour (Renwick, 2001). The effect of alkaloids on bee colony fitness and mortality has been tested in several studies (Singaravelan et al., 2005; Gegear et al., 2007; Reinhard et al., 2009; Köhler et al., 2012; Cook et al., 2013; Manson et al., 2013), which suggest that alkaloids provide benefits to weak colonies under certain circumstances. Some studies show that bees prefer nicotine and caffeine in choice experiments, perhaps because they develop dependence to these compounds (Thomson et al., 2015). Despite the possible evolutionary and ecological implications,

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the concurrent effects of floral attractiveness and bee preference on pollinator visitation have not been widely studied. These studies are important because diet has a significant effect on pathogen infections in animals and the consumption of secondary metabolites can either enhance or mitigate the severity of infections (Manson et al., 2010).

The present study investigates the influence of secondary metabolites in floral nectar on nectar preferences in pollinators by measuring the preference of *Apis mellifera carnica* for various concentrations of secondary metabolites that are known to be present in nectar (caffeine, senecionine, nicotine, and gelsemine) in artificial flowers.

MATERIAL AND METHODS

The design of the experiment followed that of Gegear et al. (2007), with several modifications. For the behavioural assay, nectar (20% sucrose solution) containing nicotine, caffeine, gelsemine, and senecionine as a free base (Sigma-Aldrich, St. Louis, USA) was used in artificial flowers.

The artificial flowers were constructed by attaching 2.5 cm wide yellow cardboard rims to the mouths of 1.5 ml microcentrifuge tubes. These flowers were weighed, filled with nectar solutions, and placed in a spiral formation on a 70 × 70 cm green Styrofoam board. Two independent overlapping concentration sets (0–0.5–1–2–5.5 and 0–0.5–2–5.5–17–50 μg ml^{-1}) were tested and later pooled for statistical evaluation. Each compound was used in triplicate per set and each set was tested in five or six independent experimental replicates, resulting in n = 15, 18, or 33 for each data point. The experimental concentration range was thus 0.5–50 μg ml^{-1}. Control flowers contained only a 20% sucrose solution.

Each flower held 1.2 ml of nectar. The green board was placed 1 m from the entrance of an outdoor hive housed in a bee-proof flight enclosure (3 × 4 × 2.5 m). The hive was housed in this enclosure for 1 week prior to the experiment, and the *Apis mellifera carnica* were supplied with pollen and honey frames during this time. No natural sources of nectar or pollen were available to the bees. At the beginning of the experiment, the bees were stimulated by dusting approximately 300 mg of pollen over the green board. The approximate volume of the solution in the control flowers was monitored over the course of the experiment, and the experiment was terminated when this volume dropped below 500 μl (which took approximately 60–90 min). The difference in the weights of the artificial flowers before and after the experiment was used to calculate the volume of nectar that was removed by the bees.

Experimental replicates were conducted twice a day, in the morning and in the afternoon, in July 2013. Between each replicate, flowers were re-filled and their positions were newly randomized. No further data filtering was applied.

Statistical analysis was done using General Linear Models followed by Dunnett's (2-sided) post-hoc multiple comparison test using the IBM SPSS Statistics (Version 20.0, 2012).

RESULTS

In the present study, honey bees preferred caffeine concentrations between 0.5–2 μg ml^{-1}, with by up to 22% higher uptake from the flowers containing 2 μg $ml^{-1}$of nectar (121.7% ± 7.0% SEM, n = 33, P = 0.045) than from the control flowers. The other alkaloids tested did not show this effect, and the attraction of all lower concentrations of the alkaloids to foraging bees was comparable to that of the control flowers (Fig. 1). In concentrations higher than 5.5 μg ml^{-1}, all compounds were repellent ($P < 0.05$). Caffeine and nicotine were slightly better tolerated than gelsemine and senecionine, which showed more significant repellence at 17 μg ml^{-1}. In the highest concentration tested (50 μg ml^{-1}), nectar uptake was approximately zero for all compounds.

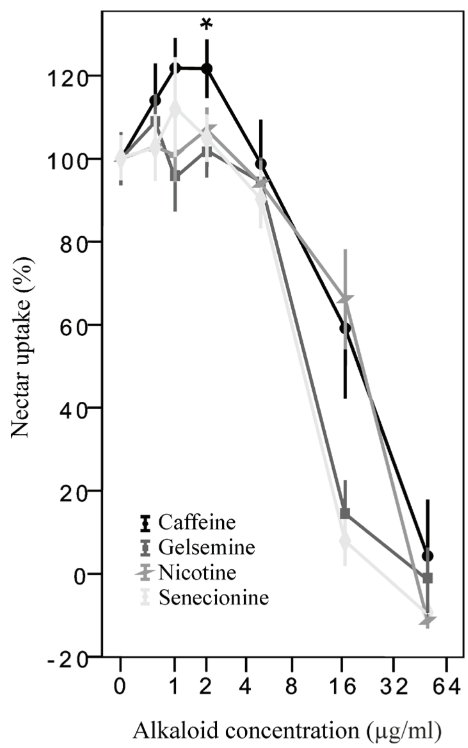

Fig. 1. Average uptake of artificial nectar containing alkaloids based on their concentration. Each point represents means of 15–33 independent replicates *statistically significant value from the control ($P < 0.05$)

DISCUSSION

Singaravelan et al. (2005) found that bees preferred 25 ppm of caffeine in artificial nectar compared to sugar solution only, which reflects the amounts naturally present in nectar of citrus flowers (11.61–94.26 ppm). In the same study, the presence of nicotine in nectar (at concentrations of 0.5 and 1 ppm) also elicited a significant feeding preference. Bees have also been shown to prefer nicotine derivatives, such as the neonicotinoids used for pest control (Kessler et al., 2015), which may have negatively affect their health.

We confirmed the preferential behaviour of bees towards caffeine but not towards nicotine. Moreover, neither of the two other alkaloids tested, senecionine and gelsemine, was preferred over the control. This shows that the preference of bees for caffeine (and for nicotine based on previous studies) is relatively specific for these alkaloids. This study differed from previous studies (such as Gegear et al., 2007) in the design and in the randomization of the flowers; we also changed the flower rims after certain experimental sets to prevent the bees from forming associations between floral colour and position and nectar properties. This was done in order to reduce the number of addicted individuals, as addictive behaviour has been previously recognized in insects (Bainton et al., 2000; Schafer, 2004). Under our experimental conditions, preference was expressed not as an increase in the frequency of visits to a flower, but rather as an increase in feed intake per visit. Interestingly, in this experiment, the preference for caffeine was observed at 10-fold lower concentrations than in the study by Singaravelan et al. (2005).

The results of this experiment support the theory that the long-term preference for caffeine and nicotine is based on addiction rather than unintentional self-medication (Gherman et al., 2014; Baracchi et al., 2015). Certain dietary elements appear to suppress the development of taste sensitivity to deterrents in insects, while the presence of specific stimulants in the diet may result in the development of dependence on these compounds (Renwick, 2001). Moreover, this suggests that taste preference depends on the presence of other compounds or concentrations simultaneously offered in nectars during experiments, or in surrounding forage. This is supported by studies in which simultaneous testing of different ranges of concentrations resulted in different preferential responses (Singaravelan et al., 2005).

To the best of our knowledge, this is the first time senecionine (as a free base) has been used in preference studies. According to our results, the presence of senecionine as a hazardous honey pollutant cannot be explained by the preferential behaviour of honey bees towards senecionine-containing flowers. Similarly, gelsemine solutions were neutral or repellent, in accordance with previous studies (Adler, Irwin, 2005; Gegear et al., 2007).

CONCLUSION

In conclusion, we tested preference for and repellence by four alkaloids in a nectar solution. We randomized the positions of flowers, which prevented the bees from memorizing the position of the preferred nectar. Data suggest that honey bees prefer caffeine not only because it elicits addictive behaviour, but also because of a taste preference. In contrast with other studies, we did not observe a preference for nicotine-containing nectars.

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Corresponding Author:

doc. Ing. Jaroslav H a v l í k , Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Kamýcká 129, 165 21 Prague 6-Suchdol, Czech Republic, phone: +420 224 382 669, e-mail: havlik@af.czu.cz