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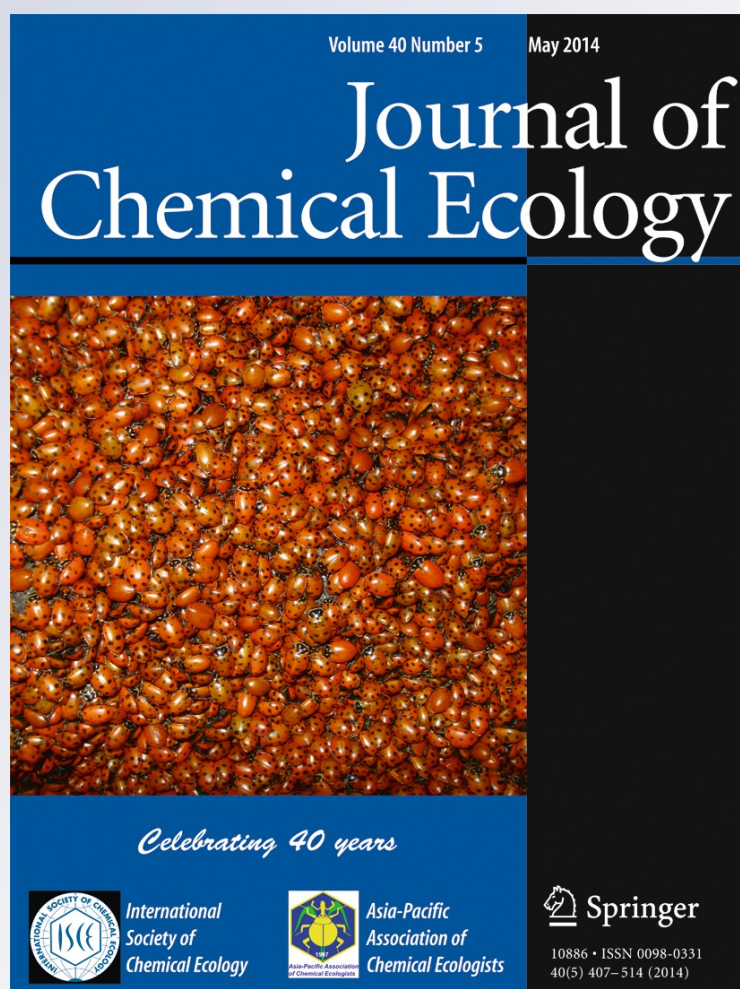
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# Nectar Minerals as Regulators of Flower Visitation in Stingless Bees and Nectar Hoarding Wasps

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**Abstract** Various nectar components have a repellent effect on flower visitors, and their adaptive advantages for the plant are not well understood. *Persea americana* (avocado) is an example of a plant that secretes nectar with repellent components. It was demonstrated that the mineral constituents of this nectar, mainly potassium and phosphate, are concentrated enough to repel honey bees, *Apis mellifera*, a pollinator often used for commercial avocado pollination. Honey bees, however, are not the natural pollinator of *P. americana*, a plant native to Central America. In order to understand the role of nectar minerals in plant–pollinator relationships, it is important to focus on the plant's interactions with its natural pollinators. Two species of stingless bees and one species of social wasp, all native to the Yucatan Peninsula, Mexico, part of the natural range of *P. americana*, were tested for their sensitivity to sugar solutions enriched with potassium and phosphate, and compared with the sensitivity of honey bees. In choice tests between control and mineral-enriched solutions, all three native species were indifferent for mineral concentrations lower than those naturally occurring in *P. americana* nectar. Repellence was expressed at concentrations near or exceeding natural concentrations. The threshold point at which native pollinators showed repellence to increasing levels of minerals was higher than that detected for honey bees. The results do

not support the hypothesis that high mineral content is attractive for native Hymenopteran pollinators; nevertheless, nectar mineral composition may still have a role in regulating flower visitors through different levels of repellency.

**Keywords** Avocado · *Nannotrigona perilampoides* · Phosphorus · Pollination · *Polybia* · Potassium · *Trigona* (= *Frieseomelitta*) *nigra* · Hymenoptera · Apoidea · Vespoidea

## Introduction

The paradox of nectar acting as both an attractant and repellent for flower visitors has gained attention in recent years. Nectar generally is attractive for a wide range of flower visitors, predominantly due to its high sugar content, but other constituents such as amino acids (Carter et al. 2006) also may attract visitors. Flower visitors, while consuming the nectar, pollinate the flower and contribute to plant fitness. Yet, within this system of mutual benefits, nectar components with repelling effects also are documented. Experiments conducted with native pollinators (Adler and Irwin 2005; Johnson et al. 2006; Kessler and Baldwin 2006) or model pollinators such as honey bees, *Apis mellifera* or *A. cerana* (Hymenoptera: Apidae) (Hagler and Buchmann 1993; Liu et al. 2004; Singaravelan et al. 2005) have exposed a range of expressions of this phenomenon. Hypotheses have been offered by Rhoades and Bergdahl (1981) and Adler (2000) for plant benefits from repellent nectar, and some have been tested experimentally (Adler and Irwin 2005; Kessler et al. 2008; Wright et al. 2013). These studies tend to focus on alkaloids, known to have a role in repelling herbivores from other plant tissues and generally regarded as pollinator repellents in nectar. Aside from alkaloids, there are other secondary nectar components whose role in attracting or repelling pollinators is not clear. It is assumed that their effects depend on concentration

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(Singaravelan et al. 2005), on interactions with other nectar components (Liu et al. 2007), or the availability of competing blooms (London-Shafir et al. 2003; Tan et al. 2012).

Minerals are among the secondary nectar components whose effect on pollinators is poorly understood. Minerals at some concentration are found in virtually all nectars, among them potassium being frequently dominant (Afik et al. 2006a; Hiebert and Calder 1983; Nicolson and W.-Worswick 1990; Waller et al. 1972). Because of their ubiquity, minerals generally are not considered pollinator repellents; to the contrary, it has been suggested that minerals, specifically calcium, are attractive to pollinators such as hummingbirds and bats (Barclay 2002; Bouchard et al. 2000; Carroll and Moore 1993). Potassium concentration varies among nectars, and generally a high potassium to sodium ratio is hypothesized to be repellent to insect pollinators (Petanidou 2007). It has been demonstrated that high potassium concentrations similar to those in the nectars of *Persea americana* (avocado) and *Allium cepa* (onion) are sufficient to repel honey bees. This has been offered as an explanation for the low preference of honey bees for these flowers in the field (Afik et al. 2006a, b; Hagler 1990; Waller et al. 1972). In avocado nectar, other minerals are found in addition to potassium, namely phosphorus, magnesium, and sulfur, all of which are suspected of having repellent action at naturally occurring concentrations (Afik et al. 2006a, 2007; Soto et al. 2013). The effect is compounded in social insects in which foragers recruit nestmates to attractive food sources. Trace components that diminish nectar attractivity translate to less rigorous dance recruitment behavior (Afik et al. 2008; Tan et al. 2012).

Although the cosmopolitan European honey bee is nowadays the main commercial avocado pollinator world-wide (Gazit and Degani 2002), it is not the natural pollinator of avocado, a plant native to Central America (Knight 2002). In order to understand the role of nectar minerals in plant-pollinator relationships, it is important to focus on natural relationships. Avocado nectar, with its naturally high mineral content, allows evaluating the role of nectar minerals in plant-pollinator relationships in a plant that depends on insect pollination for fruit set (Gazit and Degani 2002; Ish-Am and Eisikowitch 1998; Vithanage 1990). It is assumed that the natural pollinators of avocado primarily are neotropical stingless bees and honey hoarding wasps (Can-Alonzo et al. 2005; Ish-Am et al. 1999; Perez-Balam et al. 2012).

This is the first study that evaluates the behavior of native avocado flower visitors with regard to the mineral content of nectar. We tested the hypothesis that native avocado pollinators will not be repelled by high mineral content and may even be attracted to it. We included in our study two stingless bees (Apidae: Meliponini): *Trigona* (= *Frieseomelitta*) *nigra* (Cresson) and *Nannotrigona perilampoides* (Cresson), and one social wasp, *Polybia* spp. (Hymenoptera: Vespidae), all being common native visitors of avocado in Mexico (Can-

Alonzo et al. 2005; Ish-Am et al. 1999). We compared their behavior with that of exotic honey bees (*Apis mellifera* L.). In addition, we characterized mineral content of avocado nectar from trees grown in semi-natural conditions in Mexico and compared it with mineral content of avocado grown under intensive horticultural conditions in Israel (Afik et al. 2006a, 2007). This was done in order to verify that the high mineral content in avocado nectar is not an artifact of agrotechnical practices.

## Methods and Materials

**Experimental Sites** Experiments with stingless bees took place at the University of Yucatan, Merida, Mexico. Collecting nectar samples and experiments with native wasps were conducted in an avocado orchard in Hunucma, about 20 km west of Merida (21° 1' 0" N/89° 52' 0" W). Experiments were performed during February–March 2010. Experiments with honey bees were conducted at the Hebrew University of Jerusalem, Rehovot, Israel during January–February 2011.

**Nectar Analysis** Avocado nectar samples were collected from trees of the cultivar 'Antillano.' Clusters of flowers on several trees were enclosed with paper bags in advance of bee visitation. The nectar was collected using capillary tubes the following morning from male flowers or the next evening from female flowers. Nectar collected from different trees was pooled in order to get sufficient volume for mineral analysis, but maintaining the male and female samples separate. The male and female nectar samples were weighed and digested in closed vessel microwave-assisted digestion with 5 ml HNO<sub>3</sub> and 5 ml HCl diluted to a final volume of 25 ml with deionized water. Concentrations of different elements were determined simultaneously by inductively coupled plasma-optical emission spectrometry (ICP-OES) according to the United States EPA 6010B (1996) using Arcos ICP-OES (Spectro Analytical Instruments).

**Pollinator Preferences** Due to differences in biology and availability of the three species of natural pollinators tested, we developed different experimental methods for each species. In order to compare these species with a non-native pollinator, honey bees were included using two different methods following as close as possible the methods used for the native pollinators.

**In-Hive Feeding** We tested eight colonies of *Trigona* (= *Frieseomelitta*) *nigra* housed in wooden hive boxes with removable top covers. A plate of four feeders, each containing 800 µl sucrose solution, was introduced into each hive during the morning of the experiment day. Two of the feeders contained 50 % sucrose solution (w/w) and two contained

50 % sucrose solution with potassium. We monitored the feeders every 2 h, and removed them when the solution in one of the feeders was consumed (bottom of feeder exposed). We then measured the amount of solution left in each feeder. We defined the total volume consumed from the pure sucrose solution feeders as 100 % consumption, and we calculated consumption of the potassium enriched solution relative to this baseline. Each colony was tested consecutively for 4 days by comparing different potassium concentrations with non-supplemented control sugar solution.

We conducted a similar experiment with eight honey bee colonies. We used bird feeders that consisted of a 200 ml container that opened into a 4×4.7 cm dish. Two feeders were provided to each colony, one feeder containing 50 % sucrose solution and the other containing 50 % sucrose solution with potassium. We placed the feeders next to the hive entrance with the dishes inserted into the hive. The feeder containers were transparent and located outside the hive so the level of solution could be monitored. We measured the exact volume consumed from each feeder at the end of the experiment and calculated percent consumption as in the *Trigona* bees experiment.

The tested potassium concentrations were: 500, 1500, 5000, or 15000 ppm (0.016, 0.048, 0.16, or 0.48 M). Since phosphate is the second-most concentrated mineral in avocado nectar, the potassium was dissolved in the sugar solution in the form of potassium hydrogen phosphate ( $K_2HPO_4$ ). This mineral salt determined the phosphate concentrations to be: 200, 600, 2000, and 6000 ppm, (0.008, 0.024, 0.08, or 0.24 M), respectively.

**Foraging of Caged Colonies** Six colonies of *Nannotrigona perilampoides* were introduced into separated enclosures made of an iron frame covered by anti-aphid mesh (3×1.2×2 m), a single colony in each enclosure. The bees were trained to find a 5 ml feeder placed 2 m away from the hive. The experiment was started once bees were able to find the feeder unassisted. During the morning of each day of experiment, similar feeders were offered to the bees in each enclosure, half of them containing 50 % sucrose solution (w/w) and the other half containing 50 % sucrose solution enriched with potassium phosphate at a different mineral concentration each day. The number of feeders available for each colony was adjusted to the strength of the colony, ranging from two to six feeders, allowing the experiment to be terminated in about one hour. Feeders, without any unique color marks, were grouped together according to treatment in fixed positions during the experiment, adjacent to the control feeders. The mineral concentrations tested and methods for comparing consumption from the different solutions were similar to those described above for the in-hive experiments.

In addition, six honey bee colonies were tested inside separate enclosures (5×2.5×2 m, 20 mesh), each housing

one colony. The experiment was conducted similarly to the *Nannotrigona* experiment only by using two 200 ml feeders about 10 cm away from each other, one for each solution.

**Free Flying Wasps** Wasps, most probably of the species *Polybia diguetana* (Buysson) (but we cannot exclude the possibility of *P. occidentalis* (Olivier)), were commonly observed foraging on avocado flowers in the orchard (Fig. 3b). We first trained foraging wasps in forced-choice trials to visit 5 ml feeders containing sugar solution (50 % w/w), either pure or enriched with potassium phosphate (Fig. 3c). We then presented both feeders in a free-choice phase. To allow the wasps to discriminate between the feeders, we used a white and a yellow feeder. *Polybia* wasps learn quickly to associate colored feeders with reward and to discriminate between colors (Shafir 1996). All wasps experienced the white feeder first; for about half of them, this feeder contained the control solution and for the other half it contained the enriched solution. Each wasp that arrived at the feeder by itself was color marked to allow individual recognition, and when about five wasps were marked, the white feeder was replaced with a yellow feeder containing the reciprocal solution. The yellow feeder was available to the wasps for 15 min, enough time for each wasp to visit this feeder at least twice. After the forced-choice trials, the two feeders were available for the wasps for 1 h of free-choice trials. Every 15 min their relative position was rotated (left to right). All visits of each wasp to the feeders were recorded. When a wasp landed on one feeder but then moved to the other one, its preference was considered as the feeder from which it finally imbibed and not its initial landing feeder. Only wasps that performed at least eight visits were included in the data analysis. For eight wasps (1 day, two sessions), the mineral enriched solution available contained 500 ppm potassium (200 ppm phosphate), and for 16 wasps (3 days, five sessions) it contained 5000 ppm potassium (2000 ppm phosphate).

**Statistical Analysis** We used two-way ANOVAs to test the effects of bee species and potassium concentration on relative consumption of the potassium enriched solution by bees. The model also included the interaction between species and concentration and the colony effect nested within bee species. We first tested each data set for outliers using Grubbs's test (the extreme studentized deviate method). Significant outliers ( $P<0.05$ ) were found only in the caged colonies foraging experiment; three data points were removed from further analyses but are presented in the corresponding figure. Differences in consumption of different sugar solutions were tested by *t* test. Since each experiment included eight comparisons (two species and four K concentrations), we performed the Bonferroni correction and considered differences significant at  $P<0.006$ . The pattern of each wasp visit distribution to the feeders was tested for a difference from an expected

number of visits (see Results) to each feeder using a binomial test. When the observed distribution was significantly different from the expected distribution ( $P < 0.05$ ), the wasp was considered to have a preference for one of the feeders. Statistical analyses were performed using JMP 11.0 software (SAS Institute, Cary, NC, USA).

## Results

**Nectar Analysis** Mineral concentrations of nectar collected from male and female flowers were similar and resembled the concentrations measured in commercial avocado orchards in Israel (Table 1). Potassium was the predominant mineral in the nectar with a concentration of about 4500 ppm, followed by phosphorus with a concentration around 600 ppm. These results confirm that we captured the natural sensitivity of bees and wasps to environmental nectar concentrations of potassium and phosphorus.

**In-Hive Feeding** Both *T. nigra* and honey bees were insensitive to the lowest mineral concentrations, but from  $\geq 1500$  ppm potassium (600 ppm phosphorus) *Trigona nigra* was significantly less repelled than honey bees (ANOVA: K concentration\*species interaction,  $F_{3,63} = 4.2$ ,  $P = 0.01$ ; Fig. 1). *Trigona nigra* bees showed no preference among solutions up to potassium concentrations of 5000 ppm. Only at a potassium concentration of 15000 ppm did they prefer pure sugar solution ( $t_7 = 5.6$ ,  $P = 0.001$ ); even so, 60 % of the mineral-enriched solution was consumed at this high concentration. Honey bees had no preference among solutions at a

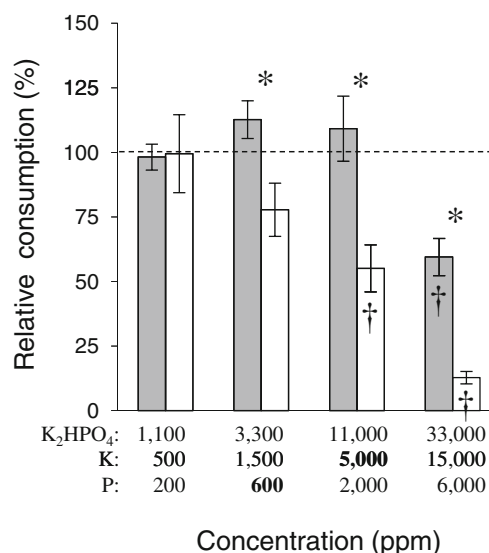
**Table 1** Concentrations (ppm) of detected minerals in avocado nectar

Location	Mexico	Mexico	Israel <sup>a</sup>	Israel <sup>b</sup>
Cultivar	'Antillano' ♂	'Antillano' ♀	'Ettinger'	'Ettinger'
T.D.S <sup>c</sup>	37 %	26 %	n.a.	30 %
K	4554.8	4533.7	3946.2	1724.4
P	568.2	643.9	511.2	301.7
Ca	247.6	164.3	<150	198.4
Mg	214.9	162.7	188.3	157.8
S	145.2	138.9	170.4	115.9
Na	34.8	39.3	53.8	52.3
Zn	16.8	16.5	<30	n.a.
B	17.6	12.8	10.8	n.a.
Mn	7.1	4.8	n.a.	n.a.
Cu	3.7	2.7	3.1	5.8
Cr	0.8	1.8	n.a.	n.a.

<sup>a</sup> Results from Afik et al. (2006a)

<sup>b</sup> Results from Afik et al. (2007)

<sup>c</sup> Total Dissolved Solids



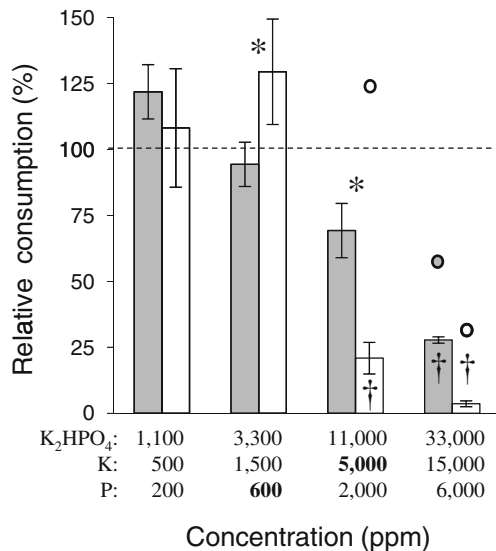
**Fig. 1** Percent consumption (mean  $\pm$  S.E.) of sugar solution enriched with potassium phosphate relative to consumption of control pure sucrose solution from feeders placed inside the hive. Gray bars represent the stingless bee *Trigona* (=Frieseomelitta) *nigra* and white bars represent honey bees. Dashed line defines baseline consumption of pure sugar solution. Concentration values in bold are natural concentrations range in avocado nectar. \* Significant differences between the two bee species (contrast test,  $P < 0.05$ ). † Significant differences between the consumption of the potassium enriched solution and the pure sugar solution ( $t$  test,  $P < 0.006$  after Bonferroni correction)

potassium concentration of 500 ppm. As the potassium concentration increased, their consumption of potassium enriched solution decreased gradually, with significant differences from the control sugar solution at the two highest concentrations (1500 ppm:  $t_7 = 2.2$ ,  $P = 0.03$ , not significant after applying the Bonferroni correction; 5000 ppm:  $t_7 = 4.9$ ,  $P = 0.002$ ; 15,000 ppm:  $t_7 = 36.7$ ,  $P < 0.001$ ).

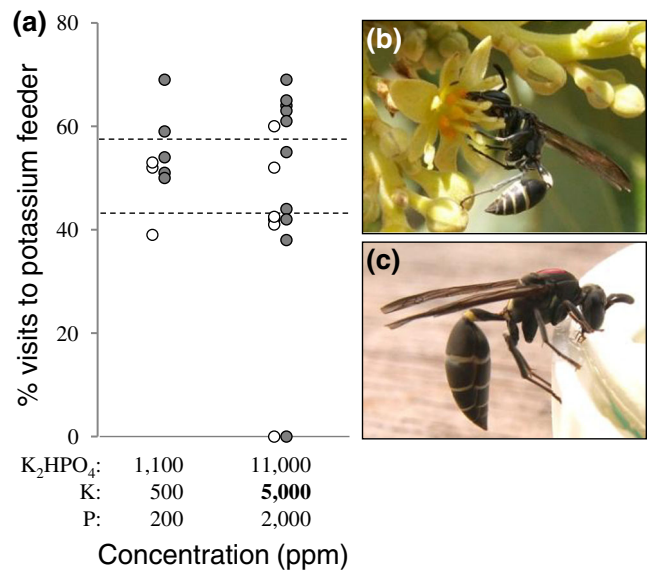
**Foraging of Caged Colonies** Honey bees tended to be more sensitive than *N. perilampoides* to high potassium concentrations, but differences were significant only for concentration of 5000 ppm after excluding outlier colonies (ANOVA: K concentration\*species interaction,  $F_{3,44} = 5.7$ ,  $P = 0.004$ ; Fig. 2). All outliers were relatively strong colonies with intensive foraging activity. At potassium concentration of 1500 ppm, the relative consumption of honey bees from the potassium enriched solution was significantly higher than that of stingless bees, even though for each species differences in consumption from control sugar solution were not significant. *Nannotrigona perilampoides* bees showed a consistent trend of decreased consumption of potassium enriched solution as potassium concentration increased. This trend ranged from a slight and non-significant preference for the mineral solution at the lowest potassium concentration up to a significant preference for the control solution at the highest concentration (500 ppm:  $t_5 = 2.1$ ,  $P = 0.09$ ; 1,500 ppm:  $t_5 = 0.7$ ,  $P > 0.05$ ;

5000 ppm:  $t_5=3.0$ ,  $P=0.03$ , not significant after Bonferroni correction; 15000 ppm:  $t_4=62.7$ ,  $P<0.001$ ). Honey bees were repelled by the two highest potassium concentrations, with negligible consumption of the 15000 ppm solution (5000 ppm:  $t_4=13.1$ ,  $P<0.001$ ; 15,000 ppm:  $t_4=91.6$ ,  $P<0.001$ ).

**Free Flying Wasps** The wasps tested in this experiment were foraging on avocado flowers in the field (Fig. 3b). Changing their feeding habits from flowers to experimental feeders (Fig. 3c) indicated that they were capable of learning quickly when the quality of reward justified it. A combined analysis of all wasp visits ( $N=639$ ) to the feeders showed a slight but significant preference for the yellow feeder (57 %,  $Z=3.5$ ,  $P<0.001$ ). Therefore, the expected visitation distribution was set to 57 %–43 % instead of 50 %–50 % visits to each feeder, and the pattern of each wasp visit distribution to the feeders was tested for a difference from the expected number of visits to each feeder calculated from her total number of visits. Performing separate analyses on the results of individual wasps suggested that no wasp showed a preference when the available alternatives were pure sugar solution or sugar solution with 500 ppm potassium. When the alternative to the pure sugar solution was sugar solution containing 5,000 ppm



**Fig. 2** Percent consumption (mean  $\pm$  S.E.) of sugar solution enriched with potassium phosphate relative to the consumption of control pure sucrose solution. Hives were kept in netted enclosures, and foragers flew to feeders placed outside the hive. *Gray bars* represent the stingless bee *Nannotrigona perilampoides* and *white bars* represent honey bees. *Dashed line* defines baseline consumption of pure sugar solution. Concentration values in *bold* are natural concentrations range in avocado nectar. \* Significant differences between the two bee species (contrast test,  $P<0.05$ ). † Significant differences between the consumption of the potassium enriched solution and the pure sugar solution ( $t$  test,  $P<0.006$  after Bonferroni correction).  $\circ$  Significant outliers (Grubbs' test  $P<0.05$ )



**Fig. 3** Foraging of *Polybia* spp. wasps. **a** The percent of visits of wasps ( $N=24$ ) to the feeder containing the mineral enriched sugar solution in a free-choice trial between mineral enriched (one of two concentrations) and control sugar solutions. *White dots* represent wasps that experienced a white potassium feeder, and *gray dots* represent yellow potassium feeder. *Upper dashed line* defines lack of preference among feeders for yellow potassium feeder and *lower line* for white potassium feeder, based on observed color preferences. *Bolded concentration values* are natural concentrations in avocado nectar. **b** A wasp foraging on *Persea americana* (avocado) flower. **c** A marked wasp feeding from the artificial feeder during the experiment

potassium, two individuals showed a clear preference for the pure sugar feeder (individual 1: 42 visits,  $Z=5.6$ ,  $P<0.001$ ; individual 2: 28 visits,  $Z=6.1$ ,  $P<0.001$ ; Fig. 3a). These two individuals usually landed directly on the pure sugar feeder, but when they landed on the mineral enriched feeder they quickly switched to the sugar feeder and consumed this solution in 100 % of their visits to the feeders.

**Discussion**

The role of nectar minerals in plant/pollinator relationships is poorly understood. Previous findings that high nectar mineral content acts as a pollinator repellent have been found in an agricultural context for a non-native pollinator visiting plants outside their natural distribution range. The aims of this study were to understand the relevance and possible outcomes of these observations for natural plant/pollinator relationships. Three possibilities can be suggested for a natural system. The first hypothesis is that the minerals that repel non-native flower visitors actually attract native pollinators and, therefore, benefit the flower (Adler 2000; Rhoades and Bergdahl 1981). A second possibility is that high mineral content deters

pollinators in the natural environment and benefits the plant by regulating visitation rates to the flowers and increasing the rates of efficient visits (Kessler and Baldwin 2006). This could be achieved, for example, by restricting visits to fewer, but more efficient, pollinator species, which are less sensitive to the high mineral content, or by restricting the amount of nectar collected in a single visit and thus increasing the number of flowers visited and subsequent cross-pollination. A third option is that nectar minerals do not affect the behavior of native pollinators, and that these observations are limited only to an agricultural ecosystem.

The first hypothesis, that nectar minerals attract native avocado pollinators, is supported by the behavior of stingless bees, which are assumed to be the natural avocado pollinators. Stingless bees are known to collect non-floral materials like mud, sweat, and human tears (Bänziger et al. 2009; Lorenzon and Matrangolo 2005). This behavior is assumed to answer a nutritional need of bees for inorganic salts, most likely sodium, potassium, and phosphorus (Roubik 1989). The composition of the honey produced by stingless bees also may give an indication of a preference for mineral rich nectar sources since its electrical conductivity, a parameter used as an indicator for honey ion content, is higher than in *Apis mellifera* honey (Souza et al. 2006; Vit et al. 1998). Therefore, it makes sense that stingless bees actively search for minerals in floral nectar, unlike honey bees (Afik et al. 2006a; Hagler 1990; Waller et al. 1972) or bumble bees (*Bombus terrestris*) (Plesser 2007), which are known to be repelled by high mineral content. Nevertheless, our results do not support the first hypothesis, since native avocado pollinators were repelled to some extent by nectar minerals. These results also contradict the third hypothesis that suggests that such repellency is limited to non-native pollinators in agricultural systems. Furthermore, even though we could not get nectar samples from trees growing under natural conditions, the similarities in nectar mineral composition between the semi-commercial orchard in Yucatan, Mexico and the intensive orchards in Israel (or even higher values in the former) suggest that high mineral content is not a result of differences in growing conditions as assumed before (Afik et al. 2006a).

Our results suggest that the mineral effect, at least in the case of potassium and phosphorus, is species- and concentration-dependent. Adding potassium and phosphorus to sugar water did not affect native pollinators at concentrations lower than those measured in avocado nectar. At mineral concentrations similar to those of avocado nectar, no effect or a slight decrease in consumption was found, but at mineral concentrations higher than those in nectar there was decreased consumption in all pollinator species. The partial repellency of native pollinators at naturally occurring concentrations is consistent with the second hypothesis. It may be that nectar minerals contribute to plant/pollinator relationships by regulating flower visits rather than attracting specific pollinators. It

remains to be shown that such regulation of pollinators contributes to plant fitness. Alternatively, nectar minerals may not have an adaptive role, but rather may be due to some plant physiological constraint.

We found differences among pollinators in their sensitivity to nectar minerals even within a single taxonomic group (Apidae). Concentrations that did not affect one species of stingless bees slightly repelled another stingless bee species and were more repulsive to honey bees. Since the generalist honey bee is not native to Central America, it is not clear who the local floral visitors are that the avocado may be repelling. One candidate group is ants that often act as nectar robbers (Galen 1983; Junker et al. 2007; Stephenson 1981), and that are commonly observed on avocado flowers, but we are unaware of any data demonstrating ant repellency in response to high nectar mineral concentration. Johnson et al. (2006) showed a selective effect in the case of the dark bitter nectar of *Aloe vryheidensis* and attributed its repulsive effect to the high content of phenolic compounds in its nectar. In contrast, phenolic compounds were suggested to attract honey bees to avocado nectar (Afik et al. 2006a) and had an attractive effect in onion nectar depending on the specific compound (Soto et al. 2013). Interestingly, both traits of aloe nectar, dark color and bitter taste, are known to be associated with high mineral content in honey (White 1992), and a significant positive correlation between mineral and phenolic content of honey has been reported (Escuredo et al. 2011). Therefore, it seems that more attention on the combined effect of nectar minerals and phenols is needed, and it cannot be dismissed that nectar minerals may play a role in the interactions between the aloe flower and its pollinators.

The current results provide further evidence for honey bee repellency from high potassium concentrations. Some studies regarding onion pollination have claimed that high potassium does not affect honey bee preference (Silva and Dean 2000; Soto et al. 2013). This was concluded since no correlation was found between potassium content in the nectars of different onion lines and the rate of bee visits to these lines. Nonetheless, the range of potassium concentration that was measured within different onion lines was relatively narrow (2790–3360 ppm, Soto et al. 2013), probably too limited to detect significant differences, and in agreement with our current results. Whereas these concentrations did not affect bees' preference between onion lines, they probably affected their behavior whether to forage on onion flowers or on competing bloom, which usually contains lower nectar potassium levels.

Another possibility that cannot be dismissed is misidentification of the target pollinator of avocado. The three native species tested in this study were selected based on previous attempts to identify the native avocado pollinators. These attempts were motivated by agricultural needs to improve commercial pollination rather than ecological interests. Since pollination of agricultural crops is dominated by social bees, it



may affect the assumption that stingless bees and social wasps are the main avocado pollinators among more than one hundred species of insects identified visiting avocado flowers in Mexico alone (Ish-Am et al. 1999). A second group of important visitors to avocado flowers are flies, mainly the families Calliphoridae, Muscidae, and Syrphidae. Flies are common visitors to avocado flowers worldwide (Australia: Vithanage 1990; Mexico: Perez-Balam et al. 2012; South Africa: Eardley and Mansell 1996). It might be that the contribution of flies to avocado pollination, especially under natural conditions, has been underestimated (Perez-Balam et al. 2012).

Non-sugar nectar components share different roles in plant/pollinator relationships and may offer both olfactory and gustatory stimuli. Some of them attract pollinators, others repel them, and many cannot be sensed by the pollinators and have no known effect on their behavior. Nectar minerals probably provide gustatory stimuli and seem to serve all these functions, being attractive to some flower visitors at certain concentrations, repulsive at higher ones, and inconsequential at others. With different effects on different pollinators at different concentrations, it is challenging to get a clear understanding of the contribution of nectar minerals to plant fitness. This study is but one step toward a deeper understanding of this issue. Kessler and Baldwin (2006) offered a memorable analogy, comparing flowers to manufacturers of soft drinks who use unique formulas to retain market share of customers. Similarly, it seems that plants such as avocado that produce mineral-rich nectar may be specializing in a niche of isotonic beverages, selecting for pollinators with distinct tastes.

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