Aphid-ant mutualism: How honeydew sugars influence the behaviour of ant scouts?

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Running headline: Honeydew sugars regulate ant foraging

Summary

Honeydew is the keystone on which ant-aphid mutualism is built. This paper investigates how each sugar identified in *Aphis fabae* Scopoli honeydew acts upon the feeding and the laying of a recruitment trail by scouts of the aphid-tending ant, *Lasius niger* Linnaeus and thus may enhance collective exploitation by their ant mutualists. *Lasius. niger* shows the following feeding preferences for honeydew sugars: melezitose = sucrose = raffinose > glucose = fructose > maltose = trehalose = melibiose = xylose. While feeding is a prerequisite to the launching of trail recruitment, the reverse is not necessarily true: not all ingested sugar solutions elicit a trail-laying behaviour among fed scouts. Trail mark laying is only triggered by raffinose, sucrose or melezitose, the latter sugar being specific to honeydew. By comparing gustatory and recruitment responses of ant foragers to sugar food sources, this study clarifies the role of honeydew composition both as a source of energy and as a mediator in ant-aphid interactions. *Lasius niger* feeding preferences can be related to the physiological suitability of each sugar –i.e. their detection by gustatory receptors as well as their ability to be digested and converted into energy. Regarding recruitment, the aphid-synthesized oligosaccharide –melezitose- could be used by ant scouts as a cue indicative of a long-lasting productive resource which is worthy of collective exploitation and defence against competitors or predators.

Key words – Ant, aphids, *Aphis fabae,* decision-making, feeding preference, honeydew sugars, *Lasius niger,,* trail recruitment

Introduction

Aphids and ants are the protagonists of one of the most studied models of mutualistic relationships in the animal kingdom. Aphids "defecate" honeydew, the sweet waste product of their sugar-rich but amino-acid-poor diet of plant sap. Following palpation by ant antennae, aphids extrude honeydew droplets without ejecting them to facilitate their collection and ingestion by ants. In return of this sugar supply, ants defend sap feeders against natural enemies and parasitoids but also provide them with hygiene by cleaning the colony from aphid exuviae and reducing risks of fungal infection (Pontin, 1959; El Ziady & Kennedy, 1956; El-Ziady, 1960; Way, 1963; Samways, 1983; Buckley, 1987; Hölldobler & Wilson, 1990; Yao *et al.*, 2000; Van Emden & Harrington, 2007).

The role of honeydew in interactions between sap feeders and ants has been long recognized. Once an aphid colony is located, a mutualistic relationship can be initiated and maintained by ants as long as the costs of aphids' protection are negligible compared to the energy provided by honeydew of adequate quality and quantity (Way, 1963; Bristow, 1991; Yao *et al.*, 2000; Woodring, 2004). Likewise, when ant colonies are faced with several aphid colonies growing concurrently in their nest surroundings, foragers will focus their tending activity and will monopolise groups of aphids which produce the most profitable honeydew, either in terms of higher volumes/number of droplets or of higher sugar content (e.g. Detrain *et al.,* 1999; Hölldobler & Wilson, 1990; Mailleux *et al.,* 2000, 2003). Honeydew composition is actually variable depending on the tended aphid species (Völkl *et al.*, 1999), on the aphid instars (Fischer *et al.*, 2002) or on the host plants inhabited by sap feeders (Völk *et al.,* 1999; Fischer & Shingleton, 2001; Fischer *et al.,* 2005). The relative amounts of some sugars such as melezitose may even change in response to interactions with ants (Fischer & Shingleton, 2001; Yao & Akimoto, 2001). In most cases, honeydew mainly contains monosaccharides (glucose and fructose) and disaccharides (maltose, sucrose) but also trisaccharides (melezitose, raffinose, erlose) as well as small amounts of amino-acids, proteins and lipids (Völkl *et al.,* 1999; Wäckers, 2000, 2001).

Since ant guarding usually increases the reproductive success of the tended sap feeders (El-Ziady & Kennedy, 1956; Flatt & Weiser, 2000; Verheggen *et al.,* 2009), evolution may have tailored honeydew composition to suit their ant mutualists. Physiological parameters such as the palatability, the fluid intake rate, the digestibility of sugar molecules as well as their suitability for the ants' survival have been studied in ants feeding on nectar and/or aphid honeydew (e.g. Josens *et al.,* 1998; Josens, 2002; Boevé & Wäckers, 2003; Roces, 2003; Heil *et al.*, 2005). Research on aphid tending ants have investigated how those physiological features can be related to feeding preferences and food amount ingested by foragers (Vander Meer *et al.*, 1995; Völkl *et al.*, 1999; Tinti & Nofre, 2001). These studies on feeding preferences have however to be completed to get an accurate overview of aphid-ant interactions since the latter are influenced by ants' sociality and colony-level responses to honeydew composition. Indeed, ant workers collect honeydew not only to sustain themselves but also to deliver collected food to a central location, the nest in which a recruitment of nestmates can be launched. Since collected honeydew represents a food source not only for the individual but also for the colony as a whole, foraging responses of ants should be designed to ingest the most suitable food for the individual but also to focus cooperation and nestmate recruitment towards the sugar resources that are worth being monopolized and defended against predators and parasitoids. Although there is a general agreement about the impact of honeydew composition on ants' foraging strategies, there is still a need to precisely quantify in controlled conditions the social response of ants facing resources differing in sugar composition. In particular, the recruitment behaviour of individual scouts feeding on different sugar molecules has never been investigated. These scouts however play a key role in the exploitation dynamics as well as in the selection of one aphid colony among several available resources: foraging patterns are shaped by the first steps of food exploitation –that is the way scouts will forage and decide to recruit nestmates depending on food profitability (Hölldobler & Wilson, 1990; Detrain *et al.*, 1999; Portha *et al.*, 2004; Detrain & Deneubourg, 2008).

In this paper, sugars present in the honeydew of *Aphis fabae* Scopoli (Stenorrhyncha, Aphidoidea), a common aphid partner of the aphid-tending ant *Lasius niger* Linnaeus (Hymenoptera, Formicidae) are identified. The feeding and recruiting responses of *L. niger* scouts to different sugar solutions are quantified in order to understand how individuals assess sugar profitability and, in some cases, organize social foraging by the laying of a recruitment trail. Ant preferences for each tested sugar will then be scaled with respect to individual gustatory responses – i.e. longer drinking times - and social responses – i.e. more intense laying of a recruitment trail. Further, what extent individual and social foraging decisions of scouts are related to chemosensory abilities of aphid-tending ants, to their digestive physiology and to the ecology of sugar producers is discussed.

Materials and methods

Ants and aphids – The common black ant, *Lasius niger* is a well-known aphid-tending species widespread in European temperate regions. Colonies were collected in Brussels and placed in plastic containers whose edges were covered with polytetrafluoroethylene (Fluon ®) to prevent them from escaping. Test tubes covered with a red transparent foil were used as laboratory rearing nests. Aqueous sucrose solution (1 M) and water filled test tubes were provided. Twice a week, dead insects (cockroaches or fruit flies) were added as protein sources. The colonies were kept in an environmentally controlled room (LD 16:8, relative humidity 65 ± 5 %, and 23 ± 2 °C). The black bean aphids, *Aphis fabae*, were mass reared on broad beans (*Vicia faba* L.) grown in 10 cm³ plastic pots filled with a mix of perlite and vermiculite (1:1) and placed in the same conditions as above.

Honeydew sugar composition – Using 0.5 µl microcapillaries, honeydew was collected from a colony of around 50 *A. fabae* unattended by *L. niger* ants. The filled capillary was immediately transferred before sugar cristallisation into a microtube containing 50 µL of milli-Q water in which honeydew was extracted. Samples were kept at -18 °C until chemical analysis. The sugar composition of *A. fabae* honeydew was measured by high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) using a Carbopac^{TM} PA-100 column (4x250 mm) and a ED40 amperometric detector (Dionex, Sunnyvale, California). This setup is adequate for mono- and polysaccharide analyses (Yao & Akimoto, 2001; Ronkart *et al.*, 2007). The elution was conducted by mixing a 0.5M NaOH solution with milli-Q water. Equilibration was conducted for 10 min with 3% NaOH solution before injection. After injection of 25 µL of sample, the concentration of the NaOH solution was increased from 3 - 59% over 25 min. Detection was operated for 25 min starting at the injection time. A control sample comprising a mix of 14 sugars of known concentration was injected prior to the analysis of the samples, and was used for sugar identification and quantification.

Sugar preferences and recruiting behaviour of Lasius niger scouts - Behaviour of *L. niger* scouts was compared when faced with equimolar water solutions (0.5 M) of the sugars that were previously identified in *A. fabae* honeydew. A single scout was allowed to reach a foraging arena on which we placed the sugar under investigation. The setup was aluminium-made and consisted in different parts (Fig. 1). (1) The access ramp (15 cm long and 1 cm wide) was placed near the nest entrance with a 45° incline and allowed scouts to reach the bridge. (2) The bridge (20 cm long and 1 cm wide) connected the ramp to the foraging arena and was covered with filter paper. (3) A 3 cm section of the bridge – located close to the ramp – could be manually removed in order to allow only a single scout to access the bridge and the foraging area. (4) The foraging arena (5X5 cm) over which we placed a bowl-shaped aluminium piece filled in with 0.4 mL of the tested sugar solution. As with the bridge, the foraging arena was entirely covered with filter paper. These papers were renewed before each test in order to remove spots of trail pheromone that could have been laid by the tested scout and that may influence feeding behaviour of subsequent ants. The following nine sugar solutions were tested: fructose, glucose, maltose, melezitose, melibiose, raffinose, sucrose, trehalose and xylose (Sigma-Aldrich, Chemie GmbH, Steinheim, Germany). Sugar solutions were made with distilled water, which was therefore used as control. Water control and sugar solutions were tested once per experimental series in a random order.

Test ant colonies were deprived of food but allowed water for 4 days. Before the start of the experiment, colonies were connected to the bridge-arena setup for 1h and sugar solutions, stored at +5 °C, were left at ambient temperature. After 1h, exploring ants, if any, were removed from the setup, filter papers were renewed and a sugar solution was placed in the middle of the foraging arena. Once a scout reached the mid-part of the bridge, the movable section of the bridge was taken away. The foraging behaviour of this scout was then recorded using the Observer® software (Noldus information Technology, version 5.0, Wageningen, The Netherlands) until it left the foraging arena and the bridge to go back to its nest. Three behavioural sequences were recorded:

1). the time spent walking on the bridge and the arena before drinking the sugar solution,

2) the time spent at the food source, touching and drinking the sugar solution, this measure being highly correlated to the amount of ingested food (Portha *et al.*, 2004),

3) the time spent walking on the bridge and the arena after having drunk the sugar solution.

With regard to recruitment, the number of trail spots laid on the bridge was assessed by observing how many times a scout bent its gaster downwards and contacted the substrate over a 6 cm bridge section on its way back to the colony. This trail-laying behaviour was used as an estimate of the ant's readiness to engage the whole colony into the exploitation of a profitable food source. Each scout having contacted the sugar solution with its mandibles or its antennae was followed until it reached the end of the bridge. Sixteen different scouts from four different colonies were tested for each sugar solution.

Results

Honeydew sugar composition

Nine sugars were identified in aphid honeydew by comparing their retention times with those of known standards: trehalose, glucose, xylose, fructose, melibiose, sucrose, melezitose, raffinose and maltose (Table 1). Eight additional peaks were also detected but could not be identified. Sucrose and fructose were the main sugars present in *A. fabae* honeydew and showed the highest concentrations (14.3 gL^{-1} and 8.1 gL^{-1}) as well as the highest molarities (around 0.04 M) among all the identified sugars. The monosaccharide glucose was also quite abundant both in terms of mass concentration (3.9 gL^{-1} respectively) and molarity (0.02 M). Only small amounts of melezitose (2.2 gL^{-1}) were found in *A. fabae* honeydew and accounted for less than 0.005 M. The remaining five sugars were present at low levels of less than 1 gL^{-1}.

Sugar preferences of Lasius .niger scouts

For each tested sugar, the time spent by scouts exploring the foraging area before reaching the sugar source was not statistically different from the water control (Dunnett's comparison with control, $P > 0.05$). This indicates that ants were unable to detect sugar solutions from a distance and that they discover them by chance. Five sugar solutions induced a significantly longer lasting feeding behaviour than water control: fructose, glucose, melezitose, raffinose and sucrose (fig. 2A; Dunnett's comparison with control, $P < 0.05$). The time spent on the foraging area after having drunk at the sugar solution was significantly lower than with water for glucose, melezitose, raffinose and sucrose (Fig. 2B: Dunnett's comparison with control, $P < 0.05$). By contrast, for the other tested sugars, ants hardly drunk at the food source and walked for a long time on the foraging area. With regard to nestmate recruitment, scouts deposited significantly more trail spots on their way back to the colony after having ingested melezitose, sucrose or raffinose (Fig. 3 Dunnett's comparison with control, $P < 0.05$). Trail-laying behaviour was occasionally observed towards fructose and glucose but levels were not statistically different from that of the water control (Fig. 3 Dunnett's comparison, $P > 0.05$).

Based on these results, one can separate honeydew sugars into three groups (Figs 2 , 3). The first group consists of those sugars that were less ingested by scouts and that did not trigger laying a recruitment trail–i.e. melibiose, xylose, trehalose and maltose.

Glucose and fructose both belong to a second group of sugars that were ingested longer than water but that did not induce a significant amount of trail-laying behaviour. Finally, in the third group are the oligosaccharides - sucrose, raffinose and melezitose - that elicit prolonged drinking behaviour as well as a pronounced trail-laying behaviour among individual scouts.

Discussion

Workers of several ant species are ubiquitous visitors to liquid carbohydrate sources, which are usually plant nectar, extrafloral nectaries or honeydew produced by aphid colonies (Hölldobler & Wilson, 1990; Engel *et al.,* 2001). The intensity as well as the persistence of interactions between ant and aphid partners are positively related with honeydew quantity and quality (Bristow, 1991; Bonser *et al.*, 1998; Völkl *et al.,* 1999; Detrain *et al.,* 1999; Mailleux *et al.,* 2000; 2003). Honeydew composition is therefore the keystone on which ant–aphid mutualism is built on. The occurrence and relative abundance of honeydew sugars are specific to the honeydew-producing species (Hendrix *et al.*, 1992), and can be used by ants to differentiate between honeydew from different phloem feeders. In the present study, sucrose, fructose and glucose make up the bulk of *A. fabae* honeydew by accounting for nearly 90% of identified sugar content. *Aphis fabae* also contains small amounts (less than 2%) of carbohydrates such as xylose and the disaccharides trehalose, melibiose, maltose. A similar composition of honeydew has been previously reported in several ant-attended aphid species (Völkl *et al.,* 1999; Fischer *et al.,* 2002, 2005). *Aphis fabae* excreta in the present study however contains smaller amounts of melezitose than usually produced by ant -attended aphids (Fischer *et al.,* 2005). It may be assumed that this low concentration of melezitose (<0.005 M) characterizes aphids unattended by ants, the production of this trisaccharide being induced only after prolonged interactions with tending ant workers (Fischer & Shingleton, 2001).

By comparing gustatory and trail-laying responses by ants tasting different sugar solutions, the present study contributes to clarify the role of honeydew sugars both as a food and as a mediator in ant-aphid interactions. To successfully use aphid honeydew as a source of energy, ants must first exhibit a positive gustatory response to its component sugars and consequently be capable of digesting and metabolizing the sugars. In addition, ant foragers must tune the level of ant-aphid interactions: scouts should increase recruitment intensity towards sugar molecules of intrinsic value or when they detect sugars that characterize food sources worth of being monopolized and defended by the ant colony.

In this study, sugars can be broadly classified into:

a) Sugars that elicit no feeding response and no recruitment
b) Sugars that are ingested by ants but that fail to elicit a recruitment
c) Sugars that are ingested and that trigger the laying of a recruitment trail

The gustatory detection and feeding behaviour of ants are likely to be correlated with the profitability of sugars in further metabolic processes, e.g. sugars being less digestible or providing less gain than the energy needed to digest them could be less preferred (Boevé & Wäckers 2003). Sugars from the first group- i.e. xylose, melibiose, trehalose and maltose- are less ingested than the others by scouts which subsequently spend more time before returning to the nest without laying a recruitment trail. Likewise, unsatiated ants provided with a too tiny sucrose droplet usually spend a longer time on the foraging area in comparison with satiated ants having fed on a very large food source (Mailleux *et al*., 2000). This strongly suggests that workers faced with those poorly exploited sugars are unsatiated and are searching for additional resources. Xylose is hardly metabolized by animal cells (Terra & Ferreira, 1994) and xylanase enzymatic activity has not been detected in ant species (Ertha *et al*., 2004). The disaccharide melibiose has to be hydrolyzed by a β-galactosidase in order to cross gut walls but the activity of this enzyme is usually weaker or even absent in ant digestive tract (Ferreira *et al.*, 1998; Boevé & Wäckers, 2003). Trehalose and maltose disaccharides are both composed of two alpha-linked glucose molecules that should be broken down by glucosidase enzymes – trehalase and maltase, respectively- to become available as energy source for insect cells (Wyatt, 1967). The occurrence, as well as the activity levels, of those two enzymes in insect digestive tracts are highly variable and remain to be investigated in aphid-tending ants (Febvay & Kermarrec, 1986; Jabbar & Mohammed, 1990; D'Ettore *et al.,* 2002; Erthal *et al.,* 2004). Here, it is assumed that the activity of gut trehalases and hence trehalose digestibility could be far lower in aphid tending ants such as *L. niger* as compared with ants feeding on insects containing high amounts of this disaccharide (e.g. *Myrmica rubra* studied by Boeve & Wäckers 2003).

The ants spent a longer time drinking at the food droplets containing sugars in groups 2 and 3. When fed, they returned quickly to the nest what strongly suggests that the nutritive needs of individual workers were met by those sugars (Mailleux *et al.*, 2000). The ingested monosaccharides, fructose and glucose are the most common sugars present not only in excreta of sap-feeders but also in floral nectars (Baker & Baker, 1982). Since these monosaccharides can cross the intestinal barrier of insects, they are readily available for metabolic processes and can be used as energy (Wyatt, 1967; Boevé & Wäckers, 2003). Glucose molecules circulating in haemolymph can also be stored as trehalose, this storage molecule providing twice the energy for respiratory needs than glucose, without altering the osmolarity (Turunen, 1985, for review and other functions of trehalose see Thompson, 2003). The ingested oligosaccharides, sucrose, melezitose and raffinose stimulate a strong feeding response similar to that induced by monosaccharides, even though these sugars have to be metabolized to hexose units by digestive enzymes before passing through the gut wall. In these three oligosaccharides, the presence of a fructose unit seems to play a key role in the acceptance and feeding response of ants. The link between a fructose and a glucose unit can be broken down by invertases which are commonly present in the lumen of ant gut (Heil *et* *al.,* 2005). In addition, these three sugars initiate a long-lasting feeding behaviour because gustatory taste receptors in aphid-tending ants express G*r64a* genes, a family of genes involved in sensitivity of sucrose taste receptors in fruit flies (Dahanukar *et al.*, 2007). In *L. niger* too, gustatory receptors would be particularly sensitive to sugars containing a sucrose unit, whereas the nature of a sugar as mono- versus oligosaccharide would be of minor importance.

While the feeding responses of ant workers can be confidently related to the chemical sensory abilities and metabolic processes, it is more difficult to relate the level of food acceptance by one individual scout to its further recruitment of nestmates. Indeed, while feeding is a prerequisite to the initiation of trail recruitment by scouts, the reverse is not necessarily true: not all ingested sugar solutions elicit a trail-laying behaviour among fed individuals. In particular, despite their well-known energetic benefit for attending ants, monosaccharides such as glucose and fructose do not induce the laying of a recruitment trail. By contrast, oligosaccharides from the third group – i.e. melezitose, sucrose and raffinose - lead to high frequencies of trail-laying behaviour among fed scouts. For these sugars that are accepted and easily metabolized by ants, the number of hexose units seems to enhance social transmission of food location to nestmates and hence favours collective exploitation of oligosaccharides with a higher energetic content. However, recruiting behaviour of *L.niger* scouts towards sugars should also find its roots in the ecology of ant-plant mutualism. In order to link the supply by producers and the demand by consumers, one should compare the sugar composition of available resources -i.e. of plant nectar vs aphid honeydew (Percival, 1961; Baker & Baker, 1982; Völkl *et al.,* 1999). Sucrose, fructose and glucose are the only sugars found in most nectar and wound sap sources. Sucrose-dominated nectars appear to be associated with long-tubed flowers protecting the nectar against most ant species while fructose- and glucose-dominated nectars are related to 'open' flowers having unprotected nectars that can be collected by ants (Percival, 1961). Hence, once ants discover monosaccharide solutions of glucose and/or fructose, they are likely to originate from nectar. Since such a food source may be highly variable in space, temporally restricted to the flowering season and overall produced in far less quantities than aphid honeydew, it seems useless for the ants to concentrate on these resources and to mobilize large numbers of nestmates by the laying of a recruitment trail. Actually, in the field, ant–nectar interactions are usually more opportunistic and different ant species regularly share the same sources (Bluthgen *et al.*, 2000; Bluthgen & Fiedler, 2004). By contrast, honeydew contains a broader spectrum of sugars including melezitose, sucrose and raffinose that trigger intense trail recruitment among foraging ants. Melezitose and raffinose are trisaccharide sugars which are synthesized by aphids through the action of gut enzymes on plant-derived sucrose (Rhodes *et al.*, 1997; Woodring *et al.*, 2006) and which are believed to be honeydew-specific as they are scarcely described in nectar or other sugar sources (Percival 1961, Baker & Baker, 1982). Honeydew oligosaccharides have a triple function in many aphids. First, they have a physiological function which is to reduce gut osmolarity and to counteract loss of water through the gut walls (Rhodes *et al.*, 1997; Woodring *et al.*, 2006). Second, in addition to a repellent action, they may reduce the longevity of aphid parasitoids feeding on a melezitose-rich honeydew namely due to its higher viscosity (Wäckers, 2000). Finally, oligosaccharides could have an ecological function which is to aggregate ants that in turn protect the aphid colony against natural enemies. In this respect ant scouts are able to detect melezitose at very low concentrations on contact (Schmidt, 1938) and respond intensively to honeydew or water solution containing this trisaccharide (Schmidt, 1938; Kiss, 1981; Völkl *et al.*, 1999; Woodring *et al.*, 2004). From the ant perspective, aphid-synthesized oligosaccharides such as melezitose or raffinose - act as signal sugars for the workers: their detection indicates the presence of aphid colonies. Due to the generally large size of aphid colonies, to their gregarious lifestyle and their often restricted mobility, those sugar producers deserve collective exploitation and monopolization by ants. The ecological meaning of those “signal” oligosaccharides may explain why they trigger an intense trail-recruitment among aphid-tending scouts.

It still remains unclear to what extent feeding preferences of ants have shaped honeydew composition, or whether ants have adjusted their feeding and recruiting behaviour to optimize the utilization of honeydew as a sugar source. Further studies comparing the gustatory and foraging responses of other ant species faced with sugar solutions as well as honeydew of ant-tended aphid species should greatly contribute to clarify the evolutionary aspects of ant -aphid mutualism.

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Figure Legends

Figure 1. Experimental setup used to study feeding and recruiting behaviour of individual scouts. (1) access ramp, (2) movable section of the bridge, (3) 20 cm-long bridge, (4) foraging arena in which tested sugar solution was placed, (5) plastic box containing the colony.

Figure 2. Relative duration (mean + SE) spent by scouts drinking sugar solution (A) and spent walking on the foraging area after feeding at the food source(B). * indicates significant differences with water control (Dunnett's test, $P<0.05$).

Figure 3. Mean number of trail marks (+ SE) laid by *Lasius niger* scouts having tasted a sugar solution.

Table 1: Concentration, molarity (Means ± SE, *n*=6) and relative abundance of identified sugars in honeydew of 50 unattended *A. fabae* individuals

Sugars	Concentrations (gL^{-1})		Molarity ($X\ 10^{-2}$)		% Identified Sugar amounts
	Mean	(±SE)	Mean	(±SE)	
Sucrose	14.29	(±1.14)	4.18	(±0.33)	47.5%
Fructose	8.07	(±1.85)	4.48	(±1.03)	26.8%
Glucose	3.92	(±1.38)	2.17	(±0.7)	13.0%
Melezitose	2.24	(±1.26)	0.44	(±0.25)	7.4%
Melibiose	0.51	(±0.10)	0.15	(±0.03)	1.7%
Xylose	0.35	(±0.06)	0.24	(±0.04)	1.2%
Raffinose	0.32	(±0.18)	0.07	(±0.04)	1.1%
Trehalose	0.28	(±0.11)	0.07	(±0.03)	0.9%
Maltose	0.11	(±0.08)	0.03	(±0.02)	0.4%

Figure 1

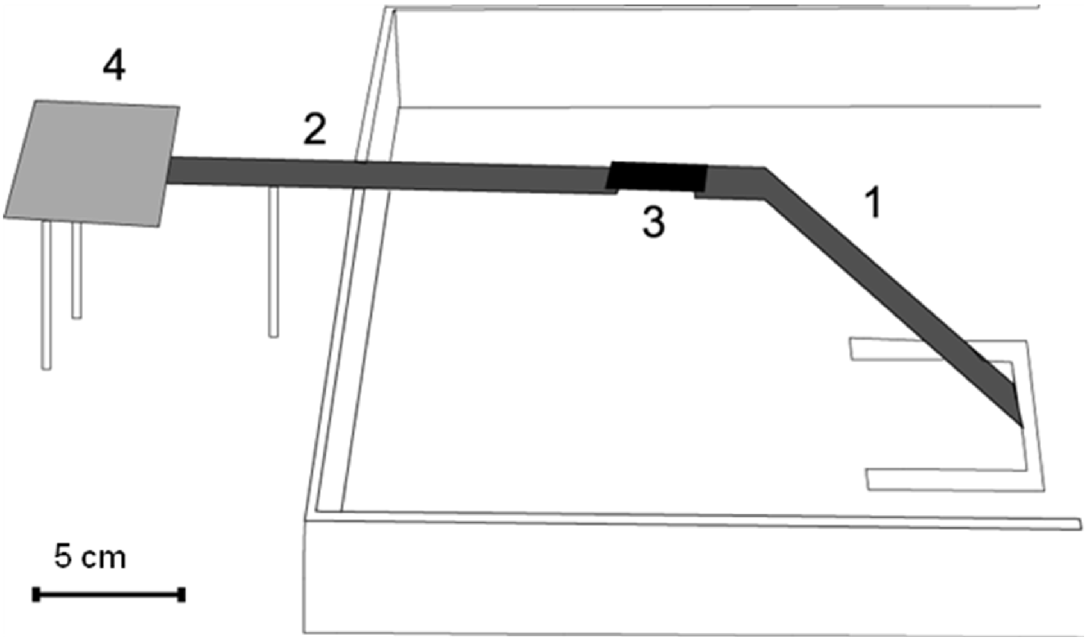
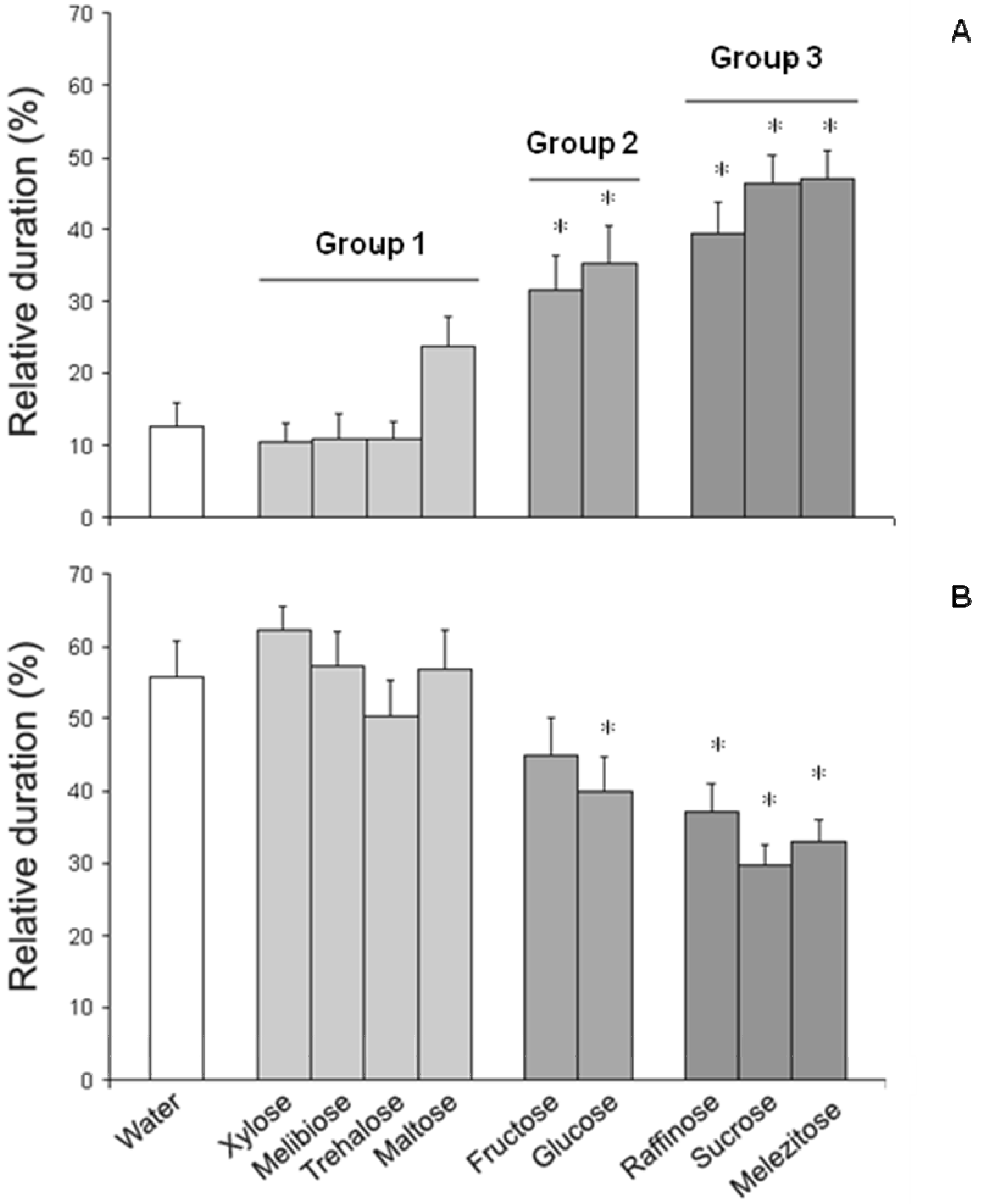
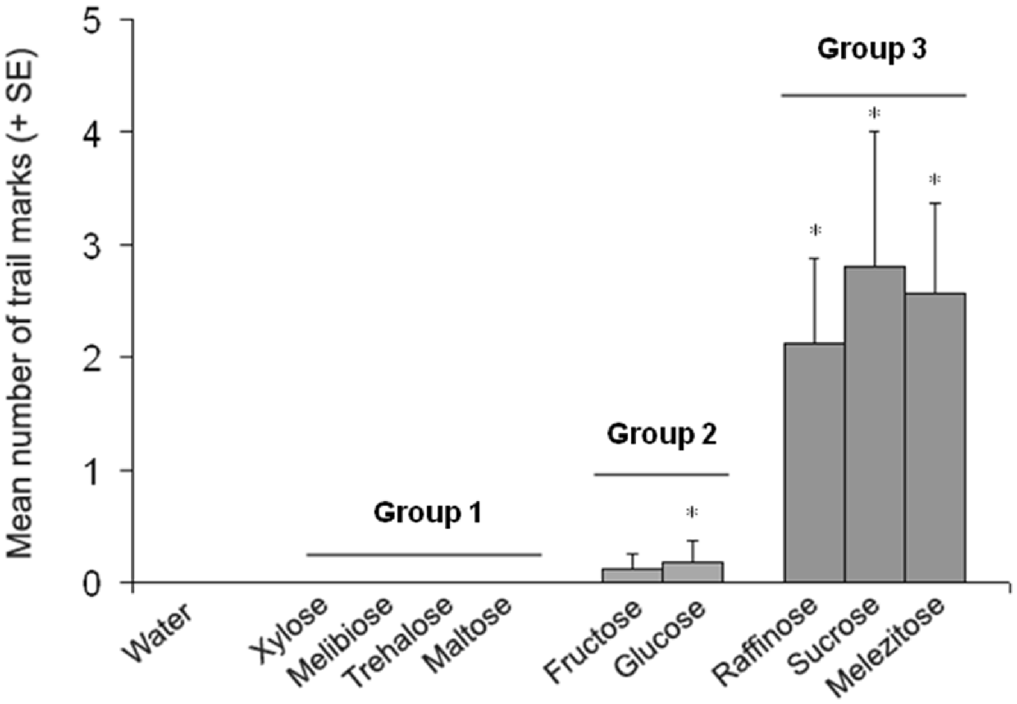


Figure 2


A
B
Relative duration (%)
0
10
20
30
40
50
60
70
Group 1
Group 2
Group 3
*
Water
Xylose
Melibiose
Trehalose
Maltose
Fructose
Glucose
Raffinose
Sucrose
Melezitose


Mean number of trail marks (+ SE)
0
1
2
3
4
5
Group 1
Group 2
Group 3
*
*
*
*
Water
Xylose
Melibiose
Trehalose
Maltose
Fructose
Glucose
Raffinose
Sucrose
Melezitose

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