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Seasonal Changes in Sugar and Amino Acid Preference in Red Wood Ants of The *Formica rufa* Group

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Abstract

Red wood ants of the *Formica rufa* group are important ecosystem engineers throughout the Northern Hemisphere with potential to be commercially produced and used as predatory agents in biological control programs. However, in order to do that, their mutualistic relationship with aphids needs to be disrupted. This may be achieved by developing artificial sugar-based solutions with a composition that makes them more attractive than aphid honeydew. The present field study investigated *Formica rufa*'s preference for several sugar and amino acid sources, as well as potential seasonal changes in these preferences. Red wood ants consistently preferred sucrose to monosaccharides and were most attracted to solutions containing an amino acid source, albeit seasonal differences were observed with regard to which amino acid sources were most preferred. Recruitment to offered sugar solutions was highest during July, when colony requirements were high, and during October, when alternative food sources were scarce. Since ant preference for sugar solution constituents seems to be species-specific and show seasonal dynamics, artificial food aimed at disrupting ant-aphid mutualisms should be tailored to individual species and seasons.

Introduction

Red wood ants (*Formica rufa* group) include a number of species with high ecological importance in temperate forests. Their presence has effects both above and below ground, playing an important role in nutrient cycling, seed dispersion and predator-prey dynamics, ultimately affecting plant and tree growth and providing benefits to a wide variety of myrmecophile species (Jurgensen et al., 2008; Stockan & Robinson, 2016).

Wood ants' diet is largely based on honeydew, which they use for metabolic maintenance as well as growth (Hölldobler & Wilson, 1990; Domisch et al., 2009). Therefore, as many other ant species, red wood ants establish mutualistic relationships with honeydew-producing insects, predominantly aphids. In exchange for honeydew, ants protect aphid colonies against (i) predators and parasitoids (Buckley, 1987; Beltrà et al., 2015), (ii) diseases (Nielsen et al., 2010) and (iii) weather (Way, 1963). Due to this mutualism, a great number of ant species can interfere with biological control of honeydew-producing pests by natural enemies (Jiggins et al., 1993; Stechmann et al., 1996; Styrsky & Eubanks 2007) and lead to increases in such pest populations (Flatt & Weisser, 2000; Stewart-Jones et al., 2008).

To obtain the protein necessary to support brood rearing, red wood ants prey opportunistically on different arthropod species, depending on their availability (Stockan & Robinson, 2016). A medium-sized nest of *Formica polyctena* has been estimated to kill eight million insects per year (Way & Khoo, 1992), and there is evidence that *Formica* ants have the potential to suppress populations of unwanted arthropods in commercial crops (Godzińska, 1986; Paulson & Akre, 1992). Furthermore, wood ant nests are easily transplanted and established in commercial orchards (Nielsen et al., 2018).

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Thus, these ants could potentially become successful biocontrol agents in horticulture when not tending harmful aphid species. In European forests, *Formica* spp. predate on several species of defoliating pests (Adlung, 1966; Skinner & Whittaker, 1981), potentially contributing to a reduction of herbivore damage on trees (Warrington & Whittaker, 1985). However, they also allow ant-tended hemipteran populations to reach damaging densities in some trees, e.g. *Fagus* (Way & Khoo, 1992). Similar patterns have been found in plantations. In an apple orchard the presence of introduced *F. polyctena* colonies led to reductions of the amount of winter moth larvae (an important pest in organic apple orchards) on ant visited trees; however, the ants also had a negative impact by increasing the number of aphids on trees (Offenberg et al., 2019). For this reason, we need strategies to disrupt ant-aphid mutualisms. This would eliminate the adverse effects derived from aphid tending and open the door to commercial production of *F. rufa* as a biological control agent, allowing us to harness the benefits derived from ant presence. Such a strategy would have an impact beyond economic benefits, as increasing the interest in wood ant production and transplantation may help improve their conservation status in nature.

Artificially provided carbohydrate solutions have successfully been used to decrease aphid tending by *Lasius niger*, resulting in significant increases in natural enemy pressure on both green apple aphids (*Aphis pomi*) and rosy apple aphids (*Dysaphis plantaginea*) (Nagy et al., 2013, 2015). Similar results were found for *Lasius grandis* in citrus orchards, further supporting the provision of artificial sugars as a viable strategy for ant management (Wäckers et al., 2017). In addition, there is evidence that sugar-fed ants may start preying on aphids that were formerly tended (Way, 1954; Offenberg, 2001). Aside from direct effects on ant-aphid dynamics, artificial sugar solutions may provide collateral benefits. In forests, the use of sugar baits on trees decreased pine weevil feeding activity on conifer seedlings by 30% (Maňák et al., 2013), possibly due to wood ants aggressively protecting their sugar sources (Maňák et al., 2015).

Due to the potential benefits of finding a sugar solution that can out-compete aphid honeydew, the field experiments in this study tested the preference of red wood ants towards different carbohydrate and amino acid sources, as previously done for *Lasius niger* (Madsen et al., 2017). Additionally, preference tests were carried out at different times during the season in order to investigate potential seasonal changes in wood ants' preferences.

Methodology

The methodology and statistical analysis in the present study are largely based on those used by Madsen et al. (2017). The sugar experiment tested *Formica*'s preference for six combinations of mono- di- and tri-saccharides (glucose, fructose, sucrose, raffinose and melezitose). Glucose, fructose and sucrose, all commonly found in aphid honeydew (Fischer & Shingleton, 2001), were used as main constituents and raffinose and melezitose were added to sucrose in small amounts to investigate their potential as attractants. The protein experiment tested *Formica*'s preference for mixtures of pure sucrose with five different amino-acid-containing substances, i.e. three mixtures of pure amino acids (AA), casein hydrolysate (Sigma-Aldrich, product 22090) and egg powder (Sigma-Aldrich, product 55871). Sucrose was selected as a carbohydrate constituent because it has been widely used in other studies and is readily accepted by many ant genera, including *Formica* (Sudd, 1985; Tinti & Nofre, 2001; Blüthgen & Fiedler, 2004).

The composition of each solution is given in Tables 1 and 2. AA mixtures were created by grouping AAs according to their ability to stimulate chemoreceptor cells, following the categorization given by Shiraishi and Kuwabara (1970). Mix A contained AAs that do not stimulate chemoreceptor cells in flies while Mix B contained those that stimulate sugar receptor cells. Mix C combined all the AAs in Mix A and B at half the concentration. Casein hydrolysate and whole egg powder provided a varied source of different peptides and AAs (Kay, 2004; Arganda et al., 2014) and, in the case of the egg powder, also fats (Dussutour & Simpson, 2008). Amino acid-only solutions, i.e. without sugar, were not included in the experiment, as they do not elicit strong ant recruitment (Blüthgen et al., 2004; personal observations).

Ten independent *F. rufa* mounds were located in forest areas in Silkeborg and Gammel Ry (Denmark). The distance between mounds in the same area varied from 25 to 250 meters, with most colonies being at least 50 meters apart (additional information on the location and size of the nests can

Table 1. Composition of solutions in the sugar preference experiment.

Solutions:	**Sugar preference experiment *g per 100 mL of water***					
Substance:	**Glucose**	**Fructose**	**Glucose + Fructose**	**Sucrose**	**Sucrose + raffinose**	**Sucrose + melezitose**
Glucose	20	–	10	–	–	–
Fructose	–	20	10	–	–	–
Sucrose	–	–	–	20	16	16
Raffinose	–	–	–	–	4	–
Melezitose	–	–	–	–	–	4

be found in Supplementary material 1). It should be noted that high intraspecific variability and hybridization make accurate identification to species level a great challenge (Stockan & Robinson, 2016), and therefore there is a chance that our colonies could be of *F. polyctena.* Experimental solutions were offered simultaneously in two separate cafeteria experiments; i.e. one for sugar preference and one for amino acid preference, carried out on the same day. Water was offered together with the baits in order to control that ants were not attracted to the different solutions due to thirst. Each set of experiments was carried out in June, July, August and October 2017.

Five milliliters of each solution were offered in small Petri dishes (35mm Ø) mounted on a larger Petri dish (135mm Ø), which served as an arena. The location of each of the solutions on the dish was randomized to control for positional bias. The feeding arena was placed in the shadow of vegetation close to a nest entrance, in areas with moderate to high ant activity. All choices within each experiment were offered simultaneously to avoid differences in discovery time. We assumed that the short distance between adjacent food sources ensured that when a forager encountered one of the baits, the ant would readily discover the others and still make a choice among all the solutions available before deciding where to drink. During the experiment, ants were observed to repeatedly visit several baits before starting to drink from one.

After offering the solutions, ants were allowed to forage freely for at least 10 minutes before the assessment of individual ant visits began. Visiting ants were counted three times, at eight to ten minutes intervals. Mean drinking time recorded for *Formica rufa* was 121 seconds (± 14,2 seconds) with a maximum drinking time of 268 seconds (Joachim Offenberg, personal communication).Thus, wood ants observed at each counting were considered different workers or workers revisiting the sugar baits after being in the nest. Since all food sources were presented in liquid form, we assume that the differences in the number of ants at each bait is similar to the differences in the amount of food collected (Kay, 2004). The total number of ants visiting each solution was summed for each colony and used as a response to analyze preferences.

Table 2. Composition of solutions in the amino acid preference experiment. All amino acids are in L – form.

Solutions:	**Amino acid preference experiment *g per 100* m*L of water***					
Substance:	**Sucrose**	**Sucrose + Mix A**	**Sucrose + Mix B**	**Sucrose + Mix C**	**Sucrose + Casein**	**Sucrose + Egg Powder**
Sucrose	20	20	20	20	20	20
Serine	–	0,25	–	0,125	–	–
Alanine	–	0,25	–	0,125	–	–
Threonine	–	0,25	–	0,125	–	–
Cysteine	–	0,25	–	0,125	–	–
Phenylalanine	–	–	0,25	0,125	–	–
Valine	–	–	0,25	0,125	–	–
Leucine	–	–	0,25	0,125	–	–
Methionine	–	–	0,25	0,125	–	–
Casein hydrolysate	–	–	–	–	1	–
Whole egg powder	–	–	–	–	–	1

Statistical model

Initially, data within each of the experiments; i.e. sugar and amino acid, was pooled for the whole season and analyzed. Subsequently, data collected in different months was analyzed separately in order to reveal potential seasonal changes in food preference. Colonies where we recorded zero or one visit during a whole experiment were excluded from the analysis.

Because different food choices were offered simultaneously, our individual counts for each solution are not independent and cannot be treated with the most common statistical methods. Therefore, data was analyzed using the Bayesian model described in Madsen et al. (2017), which estimates the distribution of possible p_i values for each food source, allowing us to estimate the probability for an ant to visit a specific source. Using the estimated distributions of probabilities, a resampling process permitted us to calculate the probability that a specific solution will be chosen over each of the alternatives.

To estimate the most likely probability for an ant to choose a specific solution we use the Dirichlet distribution (using k = 1), and the following formula:

$$\overline{p_i} = \frac{v_i + 1}{V + n},$$

where V is the total number of visits for the specific experiment, n is the number of available choices, v_i is the total number of visits to source i and p_i is the probability for a visit to source i.

To calculate the probability of solution A to be chosen over solution B, sets of p_i values (one for each of the food sources) were randomly drawn from the probability distributions previously estimated by the Dirichlet model. After 10^6 resamplings, and counting how many times solution A was preferred over B, we obtained the probability that A will be chosen over B. With this information, we assigned superscript letters to the previously estimated probabilities, in order to specify the hierarchies between each of the solutions. An R code for the resampling process is provided in Supplementary material 2. An application of the model to pollinating networks can be found in Sørensen et al. (2011) and mathematical proofs and details are given by Frigyik et al. (2010).

Results

Ant recruitment to the sugar and protein experiments varied during the season (Table 3). However, the number of ant visits was consistently higher in the protein experiments compared to sugar experiments. In all experiments, water was always among the least preferred options.

When data from the sugar experiment was pooled for the whole season, the solutions with a higher chance of being visited were sucrose and the combination of sucrose and melezitose (Table 4). Results from the resampling process (Table 5), revealed a clear preference for the disaccharide sucrose over monosaccharides. Combinations of sucrose with a trisaccharide were either less preferred than sucrose alone (raffinose) or equally preferred (melezitose). The mixture of glucose and fructose was preferred over either of the pure monosaccharide solutions. The estimated probability for an ant to choose water becomes negligible, i.e. rounded to zero, because water collection represents only a very small fraction of the total number of visits for the whole season.

Analysis of the pooled data from the protein experiment showed that all solutions containing a protein component were significantly preferred over sucrose alone. Among all the protein sources, casein was the most attractive to ants, followed by amino acid mixes B and A (Table 6).

Table 3. Total number of ant visits in each experiment by season and type of experiment. Values in brackets represent the number of colonies that contributed to the visits.

	Sugar experiment	Protein experiment
June	35 (3)	116 (6)
July	167 (8)	464 (9)
August	39 (6)	79 (4)
October	194 (10)	438 (10)

Table 4. Estimated mean value for the probability of a foraging ant to choose each of the presented solutions. Solutions with different superscript letters are significantly different at a significance level of 0.05 (see Table 5). Solutions with the same superscript letter are equally preferred.

Solution	$\bar{p}_i$
Sucrose	0.25^{a}
Sucrose + Melezitose	$0.23^{a,b}$
Sucrose + Raffinose	0.2^{b}
Glucose + Fructose	0.14^{c}
Fructose	0.1^{d}
Glucose	0.08^{d}
Water	0.00^{e}

The preference matrix resulting from the resampling process for this analysis can be found in Supplementary material 3.

Results for the individual analysis of each experiment are compiled in Table 7, which presents the seasonal dynamics of the probabilities for an ant to choose a specific solution. The accompanying matrices showing the outcome of each resampling simulation (equivalent to Table 5) can be found in Annex 3.

Table 5. Probabilities for pairwise preferences based on 10^6 simulations. Values show the probability that the solution in the row is preferred over the solution in the column at a significance level of 0.05. I.e. if values > 0.95 the row is significantly preferred over the column and if values < 0.05 the column is significantly preferred over the row.

	Glucose	Fructose	Glucose + Fructose	Sucrose	Sucrose + Raffinose	Sucrose + Melezitose
Fructose	0.750	-	-	-	-	-
Glucose + Fructose	0.991	0.954	-	-	-	-
Sucrose	1.000	1.000	1.000	-	-	-
Sucrose + Raffinose	1.000	1.000	0.985	0.036	-	-
Sucrose + Melezitose	1.000	1.000	1.000	0.292	0.893	-
Water	0.000	0.000	0.000	0.000	0.000	0.000

Table 6. Estimated mean value for the probability of a foraging ant to choose each of the presented solutions. Solutions with different superscript letters are significantly different at a significance level of 0.05 (see Table 5). Solutions with the same superscript letter are equally preferred.

Solution	p_i
Sucrose + casein	0.27^{a}
Sucrose + Mix B	0.18^{b}
Sucrose + Mix A	0.17^{b,c}
Sucrose + egg powder	0.15^{c}
Sucrose + Mix C	0.14^{c}
Sucrose	0.09^{d}
Water	0.00^{e}

Seasonal changes in sugar preference

In June, all sugar solutions except for fructose were significantly preferred over the water control. Sucrose had the highest probability of being selected, but this was not significantly different from that of the mixtures sucrose + trisaccharide or glucose + fructose. This lack of significance is likely due to the reduced sample size for this experiment. During July, all solutions with sucrose as a basic component were significantly preferred over those with monosaccharides, and the mixture of glucose and fructose was preferred over baits with only one of the two components, as seen in the analysis of the pooled data. The addition of a trisaccharide to sucrose did not make those solutions more attractive, as sucrose alone was significantly preferred over all other experimental solutions. This changed in August, where the mixture of sucrose and melezitose was significantly preferred over sucrose alone (and over monosaccharide solutions). As in July, in August there was a trend to prefer disaccharides over monosaccharides, although preference for sucrose and fructose was not significantly different (p = 0.055). In October, ants significantly preferred the mixture sucrose + melezitose over monosaccharides (but not over sucrose and sucrose + raffinose) and sucrose-based solutions over glucose.

Overall, seasonal results were similar to those obtained with the pooled data, as there was a tendency for ants to prefer sucrose-based solutions over monosaccharides, with trisaccharides sometimes increasing the attractiveness of sucrose.

Seasonal changes in protein preference

In the protein experiment in June, casein was significantly preferred over all other experimental solutions and no other significant differences were found. In July,the combination of sucrose and casein was significantly preferred over the solution containing mix C, but no significant difference was found between the former and mixes A and B. No significant difference was found in ant preference when comparing the three mixtures of single amino acids. Sucrose alone and in combination with whole egg powder were significantly disfavored, as they were the least preferred of all experimental solutions, only selected over the water control.

Table 7. Ranking of solutions from most to least preferred for each of the months in the sugar and protein experiment. In brackets, estimated mean value for the probability of a foraging ant to choose each of the presented solutions. Solutions with different superscript letters are significantly different at a significance level of 0.05. Solutions with the same superscript letter are equally preferred. *Suc* = Sucrose, *Glu* = Glucose, *Fruc* = Fructose, *Mel* = Melezitose, *Raf* = Raffinose, *Wat* = Water, *Cas* = Casein hydrolysate, *EP* = Whole Egg Powder.

	June *Solution* ($\overline{p_i}$) ($\overline{p_i}$)	**July** *Solution* ($\overline{p_i}$)($\overline{p_i}$)	**August** *Solution* ($\overline{p_i}$)($\overline{p_i}$)	**October** *Solution* ($\overline{p_i}$)($\overline{p_i}$)
Sugar experiment	*Suc* (0.28) ^{a}	*Suc* (0.30) ^{a}	*Suc + Mel* (0.39) ^{a}	*Suc + Mel* (0.22) ^{a}
	Suc + Mel (0.17) ^{ab}	*Suc + Mel* (0.21) ^{b}	*Suc + Raf* (0.26) ^{a,b}	*Suc*(0.20) ^{a,b}
	Suc + Raf (0.17) ^{ab}	*Suc + Raf* (0.20) ^{b,c}	*Suc* (0.17) ^{b,c}	*Suc + Raf* (0.17) ^{a,b}
	Glu + Fruc (0.17) ^{a,b}	*Glu + Fruc* (0.14) ^{c}	*Fruc* (0.07) ^{c,d}	*Glu + Fruc*(0.15) ^{b,c}
	Glu (0.12) ^{b}	*Fruc* (0.07) ^{d}	*Glu* (0.04) ^{d}	*Fruc*(0.14) ^{b,c}
	Fruc (0.07) ^{b,c}	*Glu* (0.06) ^{d}	*Glu + Fruc* (0.04) ^{d}	*Glu* (0.11) ^{c}
	Wat (0.02) ^{c}	*Wat* (0.01) ^{e}	*Wat* (0.02) ^{d}	*Wat* (0.00) ^{d}
Protein experiment	*Suc + Cas* (0.38) ^{a}	*Suc + Cas* (0.22) ^{a}	*Suc + Cas* (0.34) ^{a}	*Suc + Cas* (0.27) ^{a}
	Suc + Mix A (0.15) ^{b}	*Suc + Mix A* (0.21) ^{a,b}	*Suc + Mix A* (0.22) ^{a,b}	*Suc + EP* (0.24) ^{a}
	Suc + Mix B (0.13) ^{b}	*Suc + Mix B* (0.21) ^{a,b}	*Suc + Mix C* (0.19) ^{b,c}	*Suc + Mix B* (0.18) ^{b}
	Suc + Mix C (0.12) ^{b}	*Suc + Mix C* (0.17) ^{b}	*Suc + Mix B* (0.10) ^{c,d}	*Suc + Mix A* (0.12) ^{c}
	Suc + EP (0.11) ^{b}	*Suc + EP* (0.09) ^{c}	*Suc + EP* (0.07) ^{d}	*Suc + Mix C* (0.11) ^{c,d}
	Suc(0.09) ^{b}	*Suc*(0.09) ^{c}	*Suc*(0.07) ^{d}	*Suc*(0.08) ^{d}
	Wat (0.02) ^{b}	*Wat* (0.01) ^{d}	*Wat* (0.01) ^{e}	*Wat* (0.00) ^{e}

In August, casein was preferred over all other experimental solutions except for Mix A. Mix A and Mix C were similarly visited and significantly preferred over sucrose alone and sucrose mixed with whole egg powder. While Mix A was preferred over mix B, no significant difference was found between mixes B and C. The least preferred solutions were sucrose alone, mix B and whole egg powder, with no significant differences among them. In October, solutions containing casein and egg powder were preferred over all other solutions, with no significant difference between them. Amino acid mixes A and B were significantly preferred over pure sucrose. Mix B was significantly more visited than mixes A and C.

Despite the variability observed during the season, *F. rufa* workers consistently preferred solutions containing a protein source over sucrose alone and our water control had always the lowest probability of being visited.

Discussion

We have shown that wood ants prefer sucrose to monosaccharides and that adding an amino acid source increased the attractiveness of sugar solutions. Furthermore, we argue that ant preferences for specific attractants is species-specific as different preferences have been found in other ant species (see below) and that preferences for specific attractants may change over the season. These findings have implications concerning the development of sugar formulations that may be used to interrupt the mutualisms between ants and attended honeydew-producing trophobionts.

With regard to sugars, wood ants preferred sucrose to glucose and fructose. Similarly, a preference for sucrose over monosaccharides has been previously observed in *Myrmica rubra* (Boevé & Wäckers, 2003), *Lasius niger* (Völkl et al., 1999; Tinti & Nofre, 2001; Madsen et al., 2017) and ten species of tropical ants (Blüthgen & Fiedler, 2004). To our knowledge, no studies to date have described the opposite pattern for any ant species. In light of these results, the sugar component of artificial solutions aiming at breaking ant-aphid mutualisms should be based on disaccharides, primarily sucrose.

Overall, wood ants recruited more foragers in the protein experiments than the sugar ones (Poisson, Chisq = 295,7, $p = 2.2e-16$). In addition, the sucrose-only control consistently ranked among the least preferred solutions when presented along with sugar solutions that contained a protein component. Similarly, when protein components were added to sugar solutions, the vast majority of the twenty-three tropical ant species tested by Blütgen and Fiedler (2004) preferred mixtures of sucrose and amino acids over sucrose alone, and the rest were non-selective. For both *L. niger* and *S. invicta,* more workers were counted at sugar solutions containing a protein component than at sugar-only solutions (Lanza, 1991; Madsen et al., 2017). Thus, despite evidence that two tropical ant species that discriminate against specific amino acids, in favor of sugar-only artificial nectars (Lanza & Krauss, 1984), it seems that amino acid sources increase the attractiveness of sugar solutions to most ant species. This supports the idea that protein can be added to sugar to make carbohydrate solutions more attractive to ants.

Results showed that both recruitment and preferences changed during the study season. In the early summer, ants were observed exploring the pure sugar solutions and leaving them without drinking while trees in the surrounding area showed heavy ant traffic up and down tree trunks. This suggests that, at that time, ants fulfilled their sugar needs with aphid honeydew. Similarly, Sudd (1985) observed that workers of *Formica lugubris* accepted lower concentrations of sugar in the spring than they did in the summer, when aphid honeydew became available. Thus, fluctuations in availability of alternative food in their territory could explain changes in wood ant's seasonal foraging patterns. This is further supported by Kay (2004), whose field experiments found that ant species that collect extrafloral nectar or honeydew, i.e. have easy access to carbohydrates, selected a higher protein:carbohydrate ratio than species who did not.

Another reason explaining the observed seasonal variability could be changes in the demographics of the ant colony. This response has been described in fire ants *Solenopsis invicta*, where foragers adjusted food selection depending on which of the subcastes in the ant colony, e.g. reserve workers, nurses or larvae, had been starved (Sorensen et al., 1985). Similarly, colonies of *Lasius niger* with and without brood showed significantly different foraging patterns regarding sugar and protein collection (Portha, 2002). In our experiments, the highest number of visits recorded were in late July, when the needs of the colony (especially for protein) are at its peak due to high activity and brood production, and mid-October, when alternative food sources decline (Stockan & Robinson, 2016). In autumn, food availability decreases but colony demand is still high, as carbohydrates are collected and stored by young workers to use at the beginning of next season, when food is scarce (Schmidt, 1974; as quoted in Sudd. 1985). In early spring, when ant activity is resumed, workers use glands to convert stored lipids and proteins into food to feed (i) the queen and (ii) the sexual larvae resulting from the queen's "winter eggs" (Stockan & Robinson, 2016). Anticipation of this future need for nutrients in a time where alternative food is rare could explain the high recruitment observed in our experiments in October (Table 3). Lastly, laboratory experiments by Cook et al., (2010) indicated that there might be additional, season-specific cues that affect the foraging behavior of fire ants, as they found that nutrient regulation strategies were seasonally dynamic independently of colony demographics, environmental conditions or availability of food. Whether these cues are universal for all ant species remains to be investigated.

Seasonal variability was also observed regarding *Formica*'s acceptance of trisaccharides. In our experiments, raffinose and melezitose decreased the attractiveness of sucrose in July, but increased it in August. In *Lasius niger*, trisaccharides have been found to generally increase the attractiveness of sugar solutions, but while some studies found a preference for melezitose over raffinose (Völkl et al., 1999; Tinti & Nofre, 2001; Detrain et al., 2010), Madsen et al. did not (2017). Studies on several species of tropical ants did not find a preference for raffinose or melezitose over sucrose alone (Cornelius et al., 1996; Blüthgen & Fiedler, 2004). Since evidence indicates that preference for melezitose is species-specific, further studies on wood ants with larger samples sizes are needed in order to better interpret these conflicting results.

In a similar way, results on amino acid preference are also inconclusive and need further investigation. Casein was the protein source with the highest probability of being visited, both when results were pooled for the whole season and for each of the seasonal experiments. However, different solutions rank at the same preference level as casein in July, August and October (Table 7). In particular, wood ant's acceptance of egg powder should be explored, since it was clearly disfavored during summer but heavily collected (and as preferred as casein) in October (Table 7). As previously discussed, changes in food availability and colony demographics may at least partially explain this shift.

Our results indicate that a solution aiming at disrupting ant-aphid mutualism should contain carbohydrates in form of sucrose and an adequate protein source. Of all the components tested, ants showed a consistently high preference for casein, but further research is needed to gain insight on the ideal carbohydrate/protein ratio that would get transplanted red wood ants to abandon aphid tending while continuing to capture prey. Despite trisaccharides seemingly being a good choice for increasing sugar attractiveness in other ant species, e.g. *L. niger*, our results cannot support their use for *F. rufa*, and thus additional components aiming at further increasing the solution's attractiveness remain to be examined. Furthermore, and in order to corroborate the existence of a true seasonal pattern, as opposed to changes brought by chance or fluctuations in local conditions, it would be necessary to replicate this study for at least another season, preferably more.

Ideally, artificial solutions should be tailored to each ant species and to the relevant season. For example, during the late spring and summer, when aphid populations are abundant, a more nutrient-rich solution is needed in order to attract ant attention. Even after finding the ideal composition, wood ant workers might need some time to shift from foraging aphid honeydew to artificial solutions, as they exhibit high route fidelity (Stockan & Robinson, 2016). However, this strong site allegiance implies that once they start foraging on suitable artificial food, they will remain to do so for as long as it is available. Thus, the best course of action would be to start offering artificial food early in the season, when aphid populations are still absent or low in numbers. In this way, we may ensure that ants forage on artificial solutions and build up site allegiance to artificial feeders. In conclusion, offering wood ants an artificial sugar-based solution that could nutritionally outcompete honeydew, would open the door to using red wood ants as biological control agents, e.g. in fruit orchards. Besides, increased interest in optimizing commercial production of *Formica rufa* colonies could also have a positive impact in the efforts towards conservation of this key species group.

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Authors' Contributions

Madsen, NEL.: Corresponding author, experimental design, data analysis and interpretation, drafting, editing and finalization of manuscript.

Offenberg, J.: Conception of the study, substantial contributions to data interpretation, critical revision of manuscript at different stages, final approval of the version to be published. Both authors agree to be accountable for all aspects of the work.

Supplementary Materials

http://periodicos.uefs.br/index.php/sociobiology/rt/suppFiles/3760/0

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