Research Article

Received: 24 November 2023 Revised: 22 February 2024 Accepted article published: 26 February 2024 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.8049

Manipulated ants: inducing loyalty to sugar feeders with an alkaloid

Anders Lander Mogensen,[a,b†*] Laurits Bundgaard Andersen,[a,b†] Jesper Givskov Sørensen[a] and Joachim Offenberg[b]

Abstract

BACKGROUND: Wood ants are promising biocontrol agents in fruit plantations because they prey on pest insects and inhibit plant diseases. However, these ants also attend plant-feeding homopterans to harvest their honeydew secretions, thereby increasing their numbers. This problem can be solved by offering ants alternative sugar sources that are more attractive than honeydew. From natural interactions, it is known that some species manipulate mutualistic partners toward loyalty by adding alkaloids to the food they offer their mutualists. Inspired by this, the addition of alkaloids might be used to make ants loyal to artificial sugar feeders and thus used to reduce populations of ant-farmed homopterans in ant-mediated biological control. We aimed to explore whether wood ants (*Formica polyctena*) would develop a taste preference for morphine-containing sugar solutions in two-choice laboratory tests.

RESULTS: After having fed on a morphine/sugar solution for 1 week, ants showed a significant preference for morphine solutions compared with equal concentration sugar solutions without morphine. Furthermore, ants lost this preference after 6–9 days on a morphine-free diet.

CONCLUSION: The results show that wood ants react to morphine in their food, enabling chemical manipulation of their behavior, most likely through a taste preference. Thus, ants are susceptible to manipulation by mutualistic partners in natural interactions and furthermore may be manipulated artificially in biocontrol programs to avoid ant-mediated build-up of homopteran populations.

Supporting information may be found in the online version of this article.

Keywords: ant–aphid mutualism; chemical manipulation; taste preference; biological control; rapid learning

1 INTRODUCTION

In a world with an increasing population, there is a need for higher agricultural production to meet demand.[1,2] This increasing demand is mostly met by intense farming, which in turn results in a higher use of pesticides.[1] However, this approach has negative consequences for biodiversity and has been proven to damage ecosystems and non-target organisms.[3] Therefore, organic and climate-friendly solutions are attracting more and more attention as sustainable alternatives.[4,5] One move in this direction is the use of biological control.

Ants have, in many cases, proven effective as biological control agents,[6–8] because of their high abundance and generalist predatory behavior.[9,10] In addition to pest control, ants provide several other ecosystem services such as enhancing soil quality and nutrient availability, host-plant leaf nutrient uptake[11] and sometimes serving as pollinators.[10,12] Furthermore, ants also reduce plant pathogens by either eating fungal spores[13] or excreting antibiotics onto the plant when walking.[14,15] However, ants also have negative effects because they engage in trophobiotic mutualisms with harmful sap-sucking homopterans. In these interactions, homopterans excrete honeydew that the ants collect and use as a sugar source[16,17] and conversely, ants increase aphid abundance by providing a multitude of services to aphids.[16,18–20] The resulting increased aphid populations may somewhat negate the otherwise advantageous effects ants have as biocontrol agents.[21,22] However, strategies exist that can potentially solve this ant–aphid problem.

Some studies show that ants tending multiple aphids will stop tending and sometimes even start preying on the aphids providing the lowest returns in terms of honeydew quantity and quality.[23,24] Thus, the interaction with aphids might be manipulated by artificially offering additional sugar to break the ant–aphid mutualism.[25] Such manipulations have already been utilized in biological control where the feeding of ants with artificial sugar solutions counteracted the mutualism and reduced aphid

* Correspondence to: AL Mogensen, Department of Biology, Aarhus University, Ny Munkegade 114-116, Aarhus C, Denmark. E-mail: andmo@bio.au.dk

† These authors have contributed equally to this work.

a Department of Biology, Aarhus University, Aarhus, Denmark

b Department of Ecoscience, Aarhus University, Aarhus, Denmark

abundance.[26,27] This method is, however, not effective in all species-specific aphid–ant mutualistic interactions.[21,28] The wood ant *Formica polyctena* possesses a plethora of positive properties as a biological control agent. However, its mutualistic relationship with green apple aphids (*Aphis pomi*) is sometimes, but not always, broken by the supply of an artificial sugar source.[21,29] Thus, the density of aphids tended by *F. polyctena* can in some cases increase on trees both with and without this supply. A better understanding of the sugar–ant–aphid relationship and how it can be manipulated might, therefore, improve the potential of ants as biocontrol agents.

Disrupting the symbiosis between ants and aphids could potentially be accomplished by habituating ants to certain chemicals by adding these to the provided sugar solutions, and in this way induce loyalty to sugar dispensers. This is a strategy employed by some plants that manipulate their insect partners to fidelity by adding small doses of alkaloids to their nectar and extrafloral nectar rewards.[30] Laboratory studies support that ants are affected by alkaloids. Cammaerts *et al.*[31] showed the physiological effects of four different naturally occurring alkaloids (caffeine, theophylline, cocaine, and atropine) in the ant *Myrmica sabuleti*. The study found that *M. sabuleti* was susceptible to these alkaloids by showing a pronounced effect on several behavioral parameters; however, there were contrasting results on these parameters among the different alkaloids tested. In addition the ants also became habituated to one of the alkaloids used.[31] Morphine is another naturally occurring alkaloid produced in some plants.[32] A study by Entler *et al.*[33] showed that the ant *Camponotus floridanus* developed, what authors interpreted as, an addiction to morphine so strong that the ants chose a solution of morphine and water with no caloric value over a sugar solution. Furthermore, the study established that morphine-treated ants showed a significant increase in dopamine neurotransmitters compared with ants that had not been subject to morphine exposure.[33]

Both studies lay the groundwork for the use of an alkaloid–sugar solution as an alternative to pure sugar when trying to disrupt the mutualism of ants and aphids. If ants become loyal to sugar dispensers via attraction to an added alkaloid, then they will likely prefer these dispensers to honeydew, and this could open up the possibility of manipulating the ants' feeding preference.[34]

In addition to being documented from artificially manipulated laboratory studies, similar chemical manipulations also take place in naturally occurring symbiotic interactions in which species manipulate their partners to deliver higher mutualistic returns. There is increasing evidence that such manipulations take place in both plant–insect and insect–insect interactions, and that many of these manipulations are aimed at increasing the loyalty of a partner (examples are provided in the Discussion).[35–38]

In the current study, we hypothesized that wood ants would develop a taste preference for morphine in a sugar solution and therefore visit the morphine solution more than a sugar solution without morphine in a two-choice test. Furthermore, we aimed to explore whether the underlying mechanism for such manipulations is present in a proposed biocontrol agent, the European wood ant, and whether the interactions can inspire the development of more-effective biocontrol programs. Specifically, we tested whether ants developed a preference for morphine by exposing them to a morphine–sugar solution and observing whether, after a habituation period, they chose the morphine–sugar solution over a pure sugar solution. Finally, we explored whether any potential morphine preference persisted or faded over time.

2 MATERIALS AND METHODS

Wood ants (*F. polyctena*) were dug up from two ant mounds from Naaege forest, Silkeborg, Denmark and kept in a large plastic bucket (90 L) together with mound material in the form of pine needles and soil. We determined the species to be *F. polyctena* based on the hairiness.[39] The bucket was kept at room temperature (21–24 °C) and lined with paraffin oil to prevent ants from escaping. This source colony was used to produce smaller subcolonies each containing 20 adult worker ants and no ant queens. Each subcolony was kept in a plastic container (19.5 × 19.5 × 11.5 cm) with a lid containing a 10-cm diameter hole covered in fine-meshed net to prevent ants from escaping but allowing ventilation. Each container was filled with ~0.5 L of pine needles and the sides of the containers were covered with a thin layer of paraffin oil to prevent ants from escaping when the lids were off. These subcolonies were used in three experiments. In the first and second experiments (using two different sugar concentrations), we tested whether morphine-accustomed ants preferred sugar (sucrose) solutions with morphine over solutions with only sugar, and in the third experiment we tested how long the potential preference to morphine lasted.

In the first experiment (methods and results are provided in the Supporting Information, Data S1), we tested whether accustomization to a solution with 0.12 mg/mL morphine hydrochloride (Merck Life Science A/S, Søborg, Denmark. Kiros. CAS number: 52-26-6) added to a 1 M sugar solution elicited a taste preference when this solution was offered together with a 1 M sugar solution without morphine in a two-choice test. In this experiment, we did not detect any significant preference for either solution (Supporting Information, Fig. S1). We therefore decided to carry out a second experiment with a lower sugar concentration at 0.25 M (but the same morphine concentration) to increase the amount of morphine the ants had to drink to fulfill their sugar intake. In this second experiment, 18 subcolonies were randomly divided into two equally sized groups. One group was fed a 0.25 M sugar solution, and the second was fed a 0.25 M sugar solution with 0.12 mg/mL morphine hydrochloride added (Supporting Information, Fig. S2). Food solutions were offered *ad libitum* (except during starvation periods; see below), together with constant access to pure water in two 2-mL Eppendorf tubes clogged with cotton that the ants could drink through. Water and food solutions were renewed every 3 days and protein was provided in the form of one mealworm per week per subcolony. The morphine concentration was based on the recommended morphine dosage in a child scaled down to the weight of an ant.[40]

After accustomization to morphine in the morphine group, ants were subjected to two-choice tests in which they could choose between a solution with sugar only and a solution with sugar with morphine, served in two Eppendorf lids at the concentrations given above. After 7 days of feeding on a sugar or sugar–morphine diet, a two-choice test was conducted on the two diet groups. A second two-choice test was conducted again 7 days later at day 14; ants were fed the same diet in between. Each two-choice test lasted 24 h during which ants were video-recorded during the first and last 4 h of the 24-h period (Logitech Webcam C930e and Logitech BRIO #2 connected to a computer with a Logitech capture software, http://www.logitech.com/da-dk/product/capture). From these videos, within each 4-h sequence, we counted the number of ant visits to each solution for a 1-h period after their first visit to one of the solutions. The observations of the two-choice tests from the two 4-h

Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

sequences on the same replicate colonies were summed to avoid inflating the sample size by pseudo-replication. A visit was defined as an ant spending at least 5 s drinking from a lid. Before each two-choice test ants were starved for 24h to ensure plentiful feeding during the tests. The first two-choice test was done on two groups, one sugar-fed and one morphine-fed. In the second two-choice test, the sugar-fed ants from the first two-choice test became the sugar-fed morphine-exposed ants because they had been exposed to morphine previously for 24 h during the first two-choice test (this treatment is hereafter called 'Sugar-fed – ME'). To test mortality rates the number of dead ants in each subcolony was counted after the last two-choice test.

In our third experiment (Supporting Information, Fig. S3), 18 subcolonies were divided into three equally sized groups. All groups were first accustomed to morphine for 6 days by feeding on sugar with morphine (0.25 M sugar with 12 mg/mL morphine hydrochloride). Thereafter groups 1, 2 and 3 were deprived of morphine for 3, 6 and 9 days, respectively, by feeding on sugar only (0.25 M). All three groups of subcolonies were two-choice tested after the 6-day morphine-accustomization period, and after that each group was tested once more following their respective deprivation period (after 3, 6 or 9 days, respectively). Mortality rates was measured by counting the number of dead ants in each subcolony after the last two-choice test.

2.1 Statistical analysis

For all experiments the proportional visits of the two-choice tests were modeled by generalized linear models, using the binomial distributions (or quasi-binomial in experiment 2, as marked overdispersion required this) and a logit link function. The terms of these models were evaluated with the R base *anova* function using chi-squared tests.

In the second experiment, the effect of both treatment (morphine or sugar exposed) and time (1- or 2-week exposures) as well as their interaction was evaluated. In the third experiment, the choice of each group of six subcolonies after its morphine deprivation period was tested against its choice on day 0 when morphine exposure stopped.

In all experiments, because each set of subcolonies was different, statistical analyses were carried out for each group of six colonies separately, with the statistical analysis weighted by the varying number of observations in each replicate. Student's *t*-tests were conducted to evaluate the effect of the different treatments on ant mortality. All statistical tests used to analyze the data is available in the Supporting Information file, Data S2.

3 RESULTS

In the second experiment with a high morphine to sugar ratio, the analysis showed a significant effect of treatment ($\chi^2_{(1)} = 26.6$, $P = 1.3 \times 10^{-5}$), whereas no effect of exposure time or treatment by time interaction were found ($\chi^2_{(1)} = 0.13$, $P = 0.77$ and $\chi^2_{(1)} = 3.58$, $P = 0.12$, respectively). The effect of treatment was caused by a marked higher proportion of visits to morphine–sugar by morphine-fed ants compared with sugar-fed ants during both week 1 and week 2 (Fig. 1). Furthermore, ants with no previous exposure to morphine showed a preference for the pure sugar solution over the morphine–sugar solution, suggesting that the ants' first reaction to morphine is deterrence. This deterrence, however, seems to vanish after the second two-choice test when ants had been exposed to morphine for 24 h.

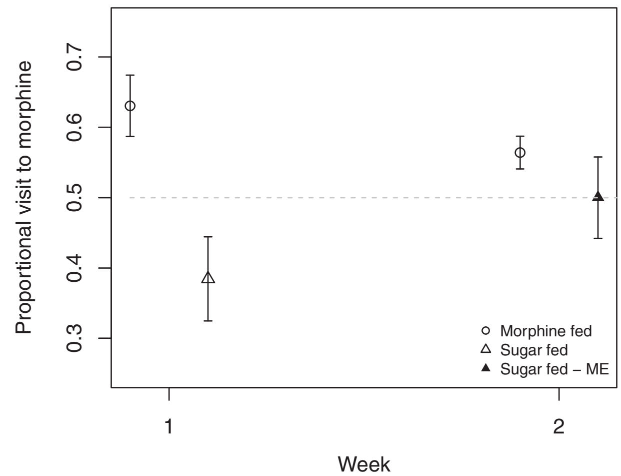

Figure 1. Second morphine experiment using the high morphine/sugar ratio. Proportional visits to morphine (morphine-fed, $n = 9$; sugar-fed, $n = 9$; sugar-fed – ME, $n = 9$), after 1 and 2 weeks respectively. Values are given as mean ± SEM are for presentation purposes based on average proportions of each replicate, whereas statistical analyses are weighted by the varying number of observations in each replicate.

In the third experiment, we tested the longevity of morphine preference in the ants. We found no difference in the proportional visits to morphine when comparing day 0 with the subcolonies tested on day 3 and day 6, respectively ($\chi^2_{(1)} = 0.08$, $P = 0.78$ and $\chi^2_{(1)} = 0.005$, $P = 0.95$). However, the subcolonies tested after 9 days without morphine showed a significantly reduced proportional visit to morphine ($\chi^2_{(1)} = 5.24$, $P = 0.02$) (Fig. 2). This suggests that morphine deterrence is reinstated after a prolonged period without access to morphine.

In none of the three experiments did we detect any statistically significant difference in mortality rates between treatments, Table S1. All data used in the three morphine experiments are available in the Supporting Information files, Data S3, S4 and S5.

4 DISCUSSION

Our study showed that after 1 week exposure to morphine, *F. polyctena* did develop a statistically significant preference for a sugar solution containing morphine (0.25 M sugar with 12 mg/mL morphine hydrochloride) over a pure sugar solution in a two-choice test. Furthermore, we showed that the morphine preference faded after 6–9 days of morphine absence. This is evidence of a mechanism that can lead to some of the chemical manipulations described from various insect and plant symbioses in nature (see below) and could be a step toward breaking the mutualistic bond between ants and aphids in biological control programs.

In our first experiment with the low morphine/sugar ratio, ants showed no preference for morphine (Supporting Information, Fig. S1), whereas in the second experiment, in which the morphine/sugar ratio was fourfold higher, we did see a preference (Fig. 1). Thus, morphine concentration is critical for the development of a preference and the potential manipulation of ants. Also, continuity was important because we observed that the preference for morphine disappeared after 6–9 days. This suggests that in a natural environment ants would be deterred by morphine; however, prolonged and continuous exposure to morphine can reverse this effect. Thus, to maintain an ant in a morphine

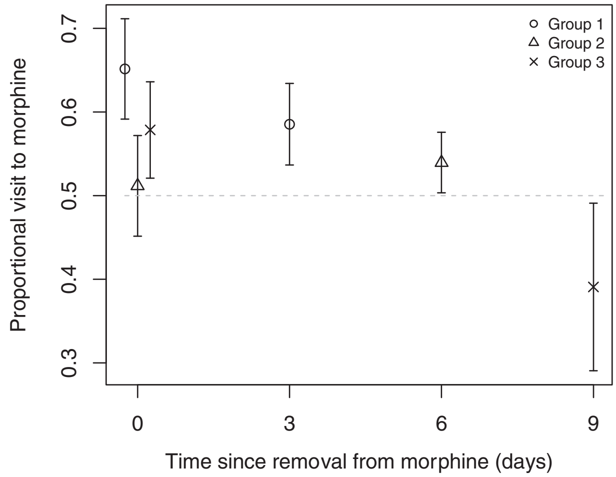

Figure 2. Third experiment. Proportional visits to morphine 0, 3, 6 and 9 days after morphine removal (with 1 week exposure to morphine before the first two-choice tests on day 0). Values given as mean ± SEM are for presentation purposes based on average proportions of each replicate, whereas statistical analyses are weighted by the varying number of observations in each replicate. Circles, triangles, and crosses represent mean ± SEM for the six subcolonies assayed 3, 6 and 9 days after the end of morphine exposure, respectively.

manipulation, a steady supply of the alkaloid is required. Whether morphine habituation and a steady intake can have negative side effects on ants remains to be tested. However, we did not observe increased mortality among the morphine-fed ants within the experimental period of this study. This supports the idea that behavioral manipulation is possible without affecting the mortality of the manipulated partner, at least in the short term.

Chemically manipulating a mutualistic partner is not new, either to biological control programs or to species interactions in the wild. In nature, chemical manipulations between interacting partners are not uncommon. In the case of ant–aphid interactions, a chemical manipulation has been described in which the aphid *Macrosiphoniella yomogicola* adds dopamine to their honeydew, which has been claimed to trigger the ant's aggressiveness after having fed on the honeydew.[36] This increased aggressiveness has been proposed as a way for aphids to lever the amount of protection that the ants provide against the aphids' natural enemies because these enemies are attacked by attending ants when they approach the aphids.[36] Also pollinating insects seem to be chemically manipulated to stay loyal to plants. For example, Baracchi *et al.*[37] showed that bumblebees increased their learning and recognition of artificial flowers when flowers contained nicotine in concentrations similar to those found in natural nectar. The higher recognition rate led to increased loyalty, foraging rate and thereby pollination.[37] Arnold *et al.*[38] later showed that bumblebees were affected similarly by another alkaloid, caffeine. It has also been speculated that ants are similarly manipulated by plants because plants secrete secondary compounds, including alkaloids, into the extrafloral nectar they offer ants; again to induce loyalty in the ant partner, which is needed by the plant for protection against herbivores and plant pathogens.[14,41] These latter types of manipulations have been reviewed by Grasso *et al.*[30] and Nepi *et al.*[42]

When considering laboratory manipulations of ants with alkaloids only few studies have been conducted. As mentioned above, Entler *et al.*[33] and Cammaerts *et al.*[31] were able to manipulate the behavior, physiology, and preference for artificial sugar feeders of *Camponotus floridanus* and *Myrmica sabuleti* by adding various alkaloids to the provided food. With our current and similar result on *F. polyctena* using morphine, the mechanism behind alkaloid manipulations seems to be a general phenomenon in ants, now found in at least three different genera across two sub-families. This points to the fact that ants can potentially be manipulated by plants and other symbiotic organisms that naturally produce these compounds, but also that such manipulations may be artificially utilized in biological control.

Although Entler *et al.*[33] argued that ants became addicted to morphine, another study shows that insects do not possess the necessary opioid receptors or neuropeptides to process opioids in a similar manner to mammals.[43] The conditioning of the ants may, therefore, result from a learned association between morphine and food, resulting in a morphine preference. In favor of the latter, Piqueret *et al.*[44] showed that *Formica fusca* could be conditioned quickly through olfactory stimuli and that their memory of the conditioning lasted up to 3 days. Furthermore, *Lasius niger* have been shown to strongly favor odors or flavors that have previously been associated with food.[45] These results fit well with the findings of this study in which ants developed a preference within a week and lost this preference between 6 and 9 days after morphine removal. This quick loss of morphine preference suggests that ants may not become addicted to morphine, but rather develop a preference for morphine because of its association with food.

We propose that alkaloid manipulations (and their chemical relatives; e.g. amines) should be considered by researchers exploring ant ecology and similarly by those working in applied myrmecology and entomology. In the latter case, we have now shown that a promising biological control agent can be manipulated to stay loyal to sugar feeders using morphine and maybe also by using other alkaloids. This opens up the use of this method to avoid problems with the build-up of aphid populations in agriculture.[25] This approach applies in cases in which ants are used actively to control pests (for example in fruit plantations), thus leaving only the positive effects of the ants.[21] However, it is also relevant in cases in which omnipresent ants that are not used in biological control programs contribute to the build-up of aphid or other honeydew-producing pest populations in various crops.[26–28,46–49] If ants become loyal to sugar dispensers via attraction to an added alkaloid, then they likely prefer these to honeydew, give up attending aphids and may even switch to aphid preying[34] rather than aphid protection, ultimately leading to lower aphid populations. Although we realize that morphine is an expensive option to use commercially, other alkaloids with similar effects could serve as a cheaper but viable alternative. However, this needs to be explored further.

It is important to note that feeding of the ants needs to start in early spring shortly after wood ants wake from their winter hibernation because at this time they will eat all available sugar sources (unpublished data) introducing the possibility of early habituation to morphine. This is further supported by the fact that ants significantly increase their visits to sugar feeders in the fall when aphid density is low, meaning that a lack of sugar will force them to choose whatever is available.[50]

Our study lays the groundwork for the use of alkaloid manipulations of ants in biological control. However, more studies are needed before the method can be applied. The intake of alkaloids can have potential effects on physiology and behavior. Any effect on linear speed, precision of reaction, response to pheromones, food consumption or aggression, as shown in Cammaerts et al.,[31] could affect ant predatory behavior and foraging activity, both of which are essential for the protection of ant-attended plants in those cases in which ants are managed for biological control. Therefore, behavioral changes due to morphine and other alkaloids need to be explored both in short-term laboratory studies and under longer term field conditions. Furthermore, any changes that might affect colony structure, phenology and survival are also important to investigate, considering that long-term colony performance is required for effective biological control.[21,51]

In conclusion, this study adds to evidence that chemical manipulations can take place in symbioses involving ants and that we may be inspired by these manipulations to potentially utilize them in biological control programs as we strive toward a green transition in agriculture.

AUTHOR CONTRIBUTIONS

ALM and LBA: Conceptualization, methodology, investigation, formal analysis, resources, writing – original draft, and writing – review & editing. JGS: Conceptualization, supervision, writing – review & editing, resources, and formal analysis. JO: Conceptualization, supervision, funding acquisition, writing – review & editing, and resources.

ACKNOWLEDGEMENTS

This study was supported by the GUDP/ICROFS Organic RDD 5 project, Open Field Biocontrol (34009-19-1562) funded under Ministry of Environment and Food of Denmark.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Carvalho FP, Agriculture, pesticides, food security and food safety. *Environ Sci Policy* **9**:685–692 (2006).
2 Rask KJ and Rask N, Economic development and food production–consumption balance: a growing global challenge. *Food Policy* **36**: 186–196 (2011).
3 Mahmood I, Imadi SR, Shazadi K, Gul A and Hakeem KR, Effects of pesticides on environment. *Plant Soil Microbes* **1**:253–269 (2016).
4 Scherr SJ and McNeely JA, Biodiversity conservation and agricultural sustainability: towards a new paradigm of 'ecoagriculture' landscapes. *Philo Transact R Soc B: Biol Sci* **363**:477–494 (2008).
5 Tahat M, Alananbeh K, Othman Y and Leskovar D, Soil health and sustainable agriculture. *Sustainability* **12**: (2020).
6 Anjos DV, Tena A, Viana-Junior AB, Carvalho RL, Torezan-Silingardi H, Del-Claro K *et al*., The effects of ants on pest control: a meta-analysis. *Proc R Soc B: Biol Sci* **289:** (2022).
7 Choate B and Drummond F, Ants as biological control agents in agricultural cropping systems. *Terrestrial Arthropod Rev* **4**:157–180 (2011).
8 Offenberg J, Review: ants as tools in sustainable agriculture. *J Appl Ecol* **52**:1197–1205 (2015).
9 Diamé L, Rey J-Y, Vayssières J-F, Grechi I, Chailleux A and Diarra K, Ants: major functional elements in fruit agro-ecosystems and biological control agents. *Sustainability* **10**:23 (2017).
10 Way MJ and Khoo KC, Role of ants in pest management. *Annu Rev Entomol* **37**:479–503 (1992).
11 Pinkalski C, Jensen K-MV, Damgaard C and Offenberg J, Foliar uptake of nitrogen from ant faecal droplets: an overlooked service to ant-plants. *J Ecol* **106**:289–295 (2018).
12 Sagers CL, Ginger SM and Evans RD, Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. *Oecologia* **123**: 582–586 (2000).
13 Thornham DG, Smith JM, Ulmar Grafe T and Federle W, Setting the trap: cleaning behaviour of *Camponotus schmitziants* increases long-term capture efficiency of their pitcher plant host, *Nepenthes bicalcarata*. *Funct Ecol* **26**:11–19 (2012).
14 Offenberg J and Damgaard C, Ants suppressing plant pathogens: a review. *Oikos* **128**:1691–1703 (2019).
15 Offenberg J, Jensen IC and Hansen RR, Combatting plant diseases with ant chemicals: a review and meta-analysis. *J Appl Ecol* **59**:25–38 (2022).
16 Buckley RC, Interactions involving plants, homoptera, and ants. *Ann Rev Ecol System* **18**:111–135 (1987).
17 Way MJ, Mutualism between ants and honeydew-producing homoptera. *Annu Rev Entomol* **8**:307–344 (1963).
18 Collins CM and Leather SR, Ant-mediated dispersal of the black willow aphid *Pterocomma salicis* L.; does the ant *Lasius Niger* L. judge aphid-host quality? *Ecol Entomol* **27**:238–241 (2002).
19 Nielsen C, Agrawal AA and Hajek AE, Ants defend aphids against lethal disease. *Biol Lett* **6**:205–208 (2010).
20 Volkl W, Aphids or their parasitoids: who actually benefits from ant-attendance? *J Anim Ecol* **61**:273 (1992).
21 Offenberg J, Nielsen JS and Damgaard C, Wood ant (*Formica polyctena*) services and disservices in a danish apple plantation. *Sociobiology* **66**:247 (2019).
22 Novgorodova TA, Ant-aphid interactions in multispecies ant communities: some ecological and ethological aspects. *Eur J Entomol* **102**: 495–501 (2005).
23 Sakata H, Density-dependent predation of the ant *Lasius Niger* (Hymenoptera: Formicidae) on two attended aphids *Lachnus tropicalis* and *Myzocallis kuricola* (Homoptera: Aphididae). *Res Popul Ecol* **37**:159–164 (1995).
24 Völkl W, Woodring J, Fischer M, Lorenz MW and Hoffmann KH, Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia* **118**:483–491 (1999).
25 Schifani E, Giannetti D and Grasso DA, Toward sustainable management of ant-hemipteran mutualism in agricultural settings: a comparison of different approaches. *Crop Prot* **175**:106468 (2024).
26 Nagy C, Cross JV and Markó V, Can artificial nectaries outcompete aphids in ant-aphid mutualism? Applying artificial sugar sources for ants to support better biological control of rosy apple aphid, *Dysaphis plantaginea* (Passerini) in apple orchards. *Crop Prot* **77**:127–138 (2015).
27 Wäckers FL, Alberola JS, Garcia-Marí F and Pekas A, Attract and distract: manipulation of a food-mediated protective mutualism enhances natural pest control. *Agr Ecosyst Environ* **246**:168–174 (2017).
28 Nagy C, Cross JV and Markó V, Sugar feeding of the common black ant, *Lasius Niger* (L.), as a possible indirect method for reducing aphid populations on apple by disturbing ant-aphid mutualism. *Biol Control* **65**:24–36 (2013).
29 Jensen IC, Hansen RR, Damgaard C and Offenberg J, Implementing wood ants in biocontrol: suppression of apple scab and reduced aphid tending. *Pest Manag Sci* **79**:2415–2422 (2023).
30 Grasso DA, Pandolfi C, Bazihizina N, Nocentini D, Nepi M and Mancuso S, Extrafloral-nectar-based partner manipulation in plant-ant relationships. *AoB Plants* **7**:7 (2015).
31 Cammaerts M-C, Rachidi Z and Gosset G, Physiological and ethological effects of caffeine, theophylline, cocaine and atropine; study using the ant *myrmica sabuleti* (Hymenoptera, Formicidae) as a biological model. *Int J Biol* **6:** (2014).
32 Fürstenberg-Hägg J, Zagrobelny M and Bak S, Plant defense against insect herbivores. *Int J Mol Sci* **14**:10242–10297 (2013).
33 Entler BV, Cannon JT and Seid MA, Morphine addiction in ants: a new model for self-administration and neurochemical analysis. *J Exp Biol* **219**:2865–2869 (2016).

34 Offenberg J, Balancing between mutualism and exploitation: the symbiotic interaction between *Lasius* ants and aphids. *Behav Ecol Sociobiol* **49**:304–310 (2001).
35 Heil M and McKey D, Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annu Rev Ecol Evol Syst* **34**:425–553 (2003).
36 Kudo T, Aonuma H and Hasegawa E, A symbiotic aphid selfishly manipulates attending ants via dopamine in honeydew. *Sci Rep* **11**:18569 (2021).
37 Baracchi D, Marples A, Jenkins AJ, Leitch AR and Chittka L, Nicotine in floral nectar pharmacologically influences bumblebee learning of floral features. *Sci Rep* **7:** (2017).
38 Arnold SEJ, Dudenhöffer J-H, Fountain MT, James KL, Hall DR, Farman DI *et al*., Bumble bees show an induced preference for flowers when primed with caffeinated nectar and a target floral odor. *Curr Biol* **31**:4127–4131.e4 (2021).
39 Seifert B, *The Ants of Central and North Europe*. Lutra, Germany, p. 408 (2018).
40 Wong C, Lau E, Palozzi L and Campbell F, Pain management in children: part 2 – a transition from codeine to morphine for moderate to severe pain in children. *Canad Pharma J* **145**:276–279.e1 (2012).
41 Rosumek FB, Silveira FA, Diniz L, Oki Y *et al*., Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* **160**: 537–549 (2009).
42 Nepi M, Grasso DA and Mancuso S, Nectar in plant-insect mutualistic relationships: from food reward to partner manipulation. *Front Plant Sci* **9**:1063 (2018).
43 Elphick MR, Mirabeau O and Larhammar D, Evolution of neuropeptide signalling systems. *J Exp Biol* **221**:jeb151092 (2018).
44 Piqueret B, Sandoz J-C and D'Ettorre P, Ants learn fast and do not forget: associative olfactory learning, memory and extinction in *Formica fusca*. *R Soc Open Sci* **6**:190778 (2019).
45 Oberhauser FB and Czaczkes TJ, Tasting the unexpected: disconfirmation of expectations leads to lower perceived food value in an invertebrate. *Biol Lett* **14**:20180440 (2018).
46 Pérez-Rodríguez J, Pekas A, Tena A and Wäckers FL, Sugar provisioning for ants enhances biological control of mealybugs in citrus. *Biol Control* **157**:104573 (2021).
47 Beltra A, Navarro-Campos C, Calabuig A, Estopa L, Wackers FL, Pekas A *et al*., Association between ants (Hymenoptera: Formicidae) and the vine mealybug (Hemiptera: Pseudococcidae) in table-grape vineyards in eastern Spain. *Pest Manag Sci* **73**:2473–2480 (2017).
48 Carabalí-Banguero DJ, Wyckhuys KAG, Montoya-Lerma J, Kondo T and Lundgren JG, Do additional sugar sources affect the degree of attendance of *Dysmicoccus brevipesby* the fire ant *Solenopsis geminata*? *Entomol Exp Appl* **148**:65–73 (2013).
49 Borbély C and Nagy C, Providing sugar sources for ants improves the biological control of aphis spp. in apple orchards. *Biol Control* **175**: 175 (2022).
50 Madsen NEL and Offenberg J, Seasonal changes in sugar and amino acid preference in red wood ants of the Formica rufa group. *Sociobiology* **67**:144 (2020).
51 Peng R and Christian K, Integrated pest management in mango orchards in the Northern Territory Australia, using the weaver ant, *Oecophylla smaragdina*, (Hymenoptera: Formicidae) as a key element. *Int J Pest Manage* **51**:149–155 (2005).