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ORIGINAL ARTICLE

Reduction in mutualistic ant aggressive behavior upon sugar supplementation

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Abstract

Mutualistic interactions between species are widespread and important for community structure and ecosystem function. In a changing environment, the proximate mechanisms that maintain mutualisms affect their stability and susceptibility to perturbation. In ant-plant mutualisms, ants defend their host plants against herbivores or competing plants in exchange for housing or food. While the phenomenon of species exchanging services for resources is well documented, how such arrangements are maintained is not. There are at least four hypothesized mechanisms through which plants use sugar to induce ant defense against herbivores. Three such hypotheses ("deficit", "fuel for foraging", "predictable rewards") predict that the appearance of a new sugar source near the host plant would increase the rate of ant attacks against herbivores, but the fourth hypothesis ("attract and distract") predicts the opposite. To examine how the mutualism between *Triplaris americana* and *Pseudomyrmex dendroicus* would be affected, we simulated the appearance of a novel sugar source at a random half of 34 *T. americana* trees with *P. dendroicus* colonies. Compared to control colonies, those with access to the sugar source were less likely to attack herbivorous insects (*Nasutitermes* sp. termites). Thus, our findings support the "attract and distract" hypothesis. We infer that this ant-plant mutualism could be destabilized by the appearance of an alternative sugar source, such as a nectar-producing plant or honeydew-excreting insect. More broadly, we conclude that the mechanisms responsible for maintaining mutualistic relationships are relevant for understanding how ecological communities are affected by environmental change.

Abstract in Spanish is available with online material.

KEYWORDS
ant behavior, ant-plant mutualism, domatia, mutualism maintenance, Peruvian Amazon, *Pseudomyrmex dendroicus*, symbiosis, *Triplaris americana*

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1 | INTRODUCTION

Mutualistic associations between ants and plants are among the most frequently studied species interactions (Bronstein, 1998; Bronstein, 2021; Mayer et al., 2014). Generally, both exhibit behaviors or morphological characteristics that benefit their symbiotic partner. The host plant provides the ant colony with shelter, food, or both (Beattie, 1989). Shelter is typically in the form of specialized chambers (domatia) and food can be provided directly in the form of nectar from extrafloral nectaries (EFNs) and/or food bodies, or indirectly through honeydew excreted by hemipteran scale insects that live inside the plants domatia and feed on phloem (Bischof et al., 2013; Blatrix et al., 2013; Fischer et al., 2002). In exchange, the ants protect their host plant from herbivores, bacterial pathogens, or encroaching vegetation (González-Teuber et al., 2014; Heil & Mckey 2003). However, ant-plant mutualistic relationships can involve complex tradeoffs that are not always obvious (Styrsky & Eubanks, 2006). Ant-plant mutualisms contribute in important ways to community structure (Bronstein, 2021), and thus their disruption or collapse could have deleterious consequences for the entire community (Montesinos-Navarro et al., 2017).

Substantial variation exists both within and between ant-plant mutualistic dyads in the degree to which host plants benefit from their ant symbionts, and vice versa (Bronstein, 1998, 2021; Díaz-Castelazo et al., 2013). Among the proposed sources of variability are the quantity and quality of food provided by the host plant, which can affect the degree to which the ants protect the plants from competitors or predators (Grasso et al., 2015; Janzen, 1985). Experimental manipulations of the amount and chemical composition of food accessible to ants have been shown to affect ant behavior and colony size (Fagundes et al., 2017; Grasso et al., 2015; Heil et al., 2001; Nepi et al., 2018). However, not all ant-plant systems respond in the same ways, and there are multiple competing hypotheses regarding the underlying proximate mechanisms.

Most hypotheses regarding the proximate mechanisms of ant-plant mutualisms are based on the idea that the chemical composition of the food provided by host plants (directly or through scale insects) encourages the ant symbionts to kill herbivorous insects. According to the "deficit" hypothesis, foods rich in carbohydrates, such as nectar and honeydew, create a nutritional imbalance to which ants respond by harvesting protein-rich prey (Ness et al., 2009). Under the "fuel-for-foraging" hypothesis, carbohydrate-rich foods support the metabolic demands of ant predatory behavior and defense of the plant against herbivores (Carroll & Janzen, 1973; Davidson, 1998; Ness et al., 2009). The "predictable rewards" hypothesis is based on the idea that as foraging ants traverse the host plant to harvest nectar or honeydew they also encounter and kill herbivorous insects (Schifani et al., 2020; Smiley, 1986). While the proposed mechanisms differ, all three of these hypotheses predict that placing a sugar source on or near a host plant should increase the level of protection it receives from the ant colony against herbivorous insects. Several studies have provided support for this prediction. For example, Ness et al. (2009) found that artificially increasing the carbohydrates available to ants increased the rate at which they attacked simulated herbivorous intruders (Lepidoptera larvae). Pringle et al. (2011) found that ants given access to a sugar solution similar in concentration to honeydew were more likely to attack Lepidoptera larvae than those given a dilute sugar solution. Likewise, Grover et al. (2007) showed that ants deprived of carbohydrates exhibited less aggression toward conspecific intruders.

However, the "attract and distract" hypothesis makes the opposite prediction and has also received empirical support (Wäckers et al., 2017). Under this hypothesis, the appearance of an alternative sugar source would draw ants away from the host plants EFNs or scale insects, leaving the plant less protected (Wäckers et al., 2017). Some facultatively myrmecophilous Lepidoptera larvae appear to employ "attract and distract" as a foraging tactic: *Synargis calyce* caterpillars compete for ants with the host plant EFNs, by secreting a substance similar to nectar, and then feed on the flower buds while receiving protection from the ants against parasitoids (Alves-Silva et al., 2018). Attract and distract has also been used, with some success, to disrupt mutualistic associations between ants and honeydew-producing crop pests (Correa et al., 2023; Parrilli et al., 2021; Pérez-Rodríguez et al., 2021; Schifani et al., 2024; Wäckers et al., 2017).

Here, we present the results of a sugar supplementation experiment designed to probe the proximate mechanisms underlying the mutualistic association between the plant species *Triplaris americana* and its obligate ant symbiont *Pseudomyrmex dendroicus* (Forel, 1904) at a lowland rainforest site in the Peruvian Amazon (Figure 1a). Abundant throughout lowland riverine habitats of Central and South American tropical forest (Brandbyge, 1986), *T. americana* is a fast-growing pioneer species that appears to rely almost exclusively on ants for defense against herbivory instead of investing in costly chemical or physical defenses (de Melo Teles E Gomes et al. 2023). *Pseudomyrmex dendroicus* is a member of a Neotropical clade of arboreal ant symbionts that aggressively protect their host plants (Ward, 1999), primarily by eliminating herbivores (Sanchez & Bellota, 2015). Despite being widely distributed across the western Amazon basin, *P. dendroicus* is highly host-plant specific, only colonizing *T. americana*, even where other species of *Triplaris* are available (Sanchez & Bellota, 2015; Weir et al., 2012). Experiments with leaf cuticular extracts have shown that the ants can distinguish between *T. americana* and other species of plants using chemical cues alone (Weir et al., 2012). Workers actively patrol the host plant, removing encroaching vegetation and herbivorous intruders (Sanchez & Bellota, 2015). The plant provides housing for the ant colony, allowing the tending of scale insects (*Coccoidea*), which feed on the plants phloem and excrete honeydew – a food source for the ants (Davidson & Mckey, 1993; Ward, 1999). Under the "deficit", "fuel for foraging", and "predictable rewards" hypotheses, supplemental sugar would provide the ants with more incentive, energy, or opportunities to defend the host plant against herbivorous insects and strengthen the mutualism. Alternatively, under the "attract and distract" hypothesis, supplemental sugar would reduce the frequency with which the ants attack herbivorous insects, and thus weaken the mutualism. To the best of our knowledge, this is the first experimental test of these hypotheses in a *Triplaris-Pseudomyrmex* species pair.

2 | METHODS

2.1 | Study area

This study was conducted in lowland forest floodplain at Cocha Cashu Biological Station (11°54S, 71°22W), located along the Manú river in Manú National Park in southeastern Peru, under permit No 06-2023-SERNANP-JEF from Servicio Nacional de Áreas Naturales Protegidas por el Estado.

2.2 | Sugar supplementation experiment

In total, we studied 34 *T. americana* trees and their *P. dendroicus* symbionts. To achieve a balanced design and to avoid potential differences between treatments in microclimate, we randomly assigned *T. americana* trees between 1 and 5 m in height with *P. dendroicus* colonies to sugar-supplemented or control treatments (17 trees per treatment) (Figure 1a). Trees separated by >2 m ($N = 24$) were assigned a treatment in a randomly alternating fashion. Ten of the trees we studied occurred in small patches of 2–3 trees <2 m apart, thus we assigned all the trees in a patch to the same treatment. Of the four patches in our study two received supplemental sugar and two were controls.

For the supplemental sugar source, we used hard candies (Jolly Rancher™, Cortex, CO, USA), which are corn-syrup based and thus mostly glucose. Most previous sugar supplementation experiments on plant-ant mutualisms have used sucrose. Our decision to use glucose was based on the materials available at this remote field station. We prepared the supplemental sugar by crushing the hard candies (mixing flavors to avoid potential flavor effects). For the supplemental sugar treatment, we placed 2-gram portions of candy at the base of the tree in an apparatus designed to prevent rain and debris from falling onto the supplemental sugar (Figure 1c). The crushed candies quickly melted to a liquid due to high heat and humidity. The supplemental sugar was replenished daily. Toward the end of the experiment, we ran out of hard candy and used powdered corn syrup mixed with water as the supplemental sugar source at eight sites (Six trees for the duration of the experiment and two trees for the final 2 days of their experiment).

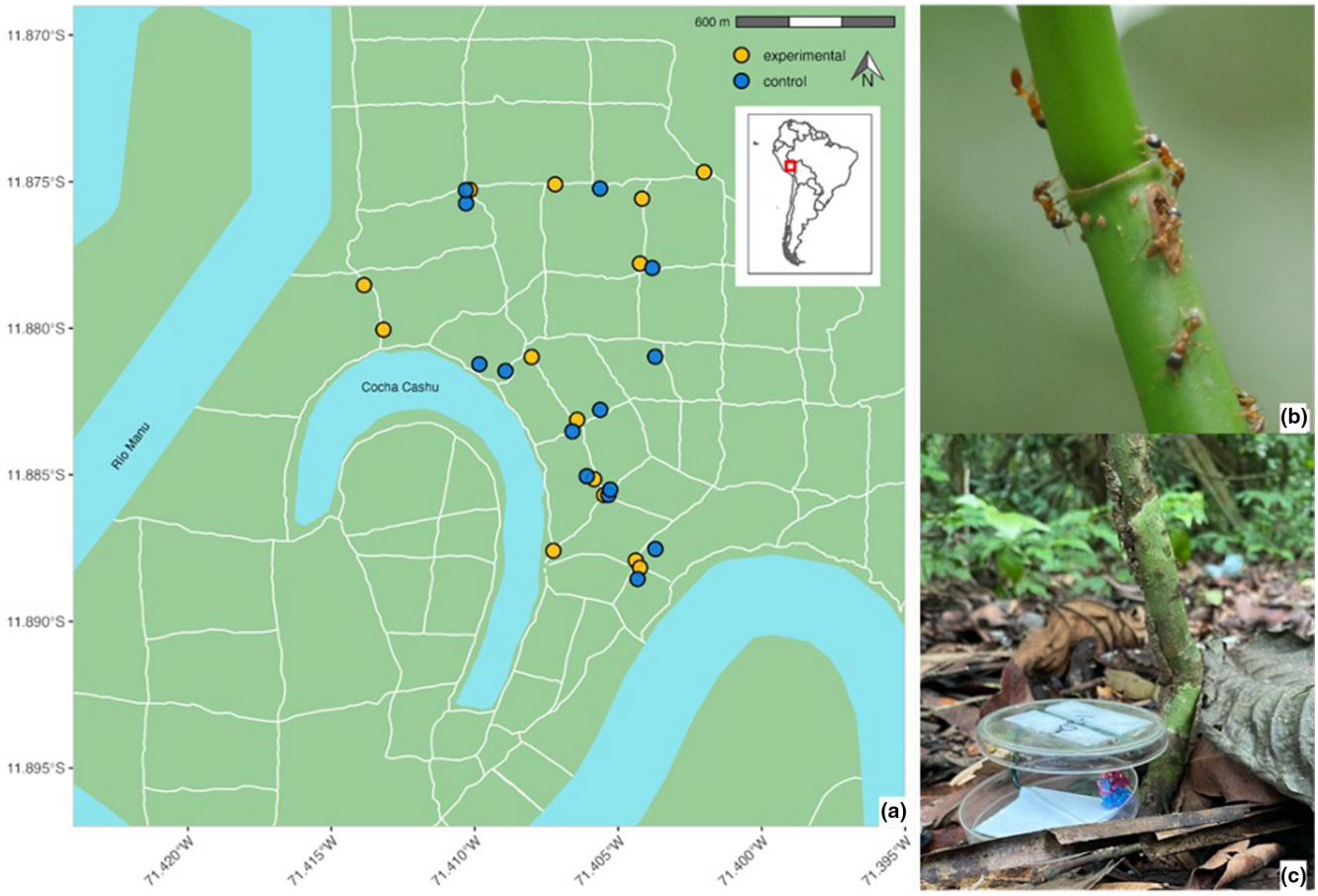


FIGURE 1 (a) Map of study sites. Locations are marked in yellow or blue to represent the experimental (sugar supplementation) and control sites, respectively. Experimental and control sites were selected in groupings to ensure unbiased sampling. (b) *P. dendroicus* colony inhabiting *T. americana* domatia. *P. dendroicus* live inside the hollowed out stems of the plant, tending to mealybugs for their honeydew secretions. (c) The sugar source was provided at the base of each experimental plant in a covered petri dish to minimize sugar loss during rainfall. Sugar was added daily to maintain a constant source for the duration of the experiment.

2.3 | Ant aggression and activity

We refer to ants attacking other insects as "aggression", whether the insects were killed or expelled from the plant. To quantify aggression, we used forceps to place a termite (Nasutitermes sp.) on the center of a *T. americana* leaf to simulate an herbivorous intruder and monitored the colonys response (following Vidal et al., 2016). All termites used in the experiment were nasute soldiers from a single arboreal nest and were collected on the same day that they were used. A different termite was used for each intruder test. Previous field experiments on *Pseudomyrmex-Triplaris* and other ant-plant mutualisms also used termites to assess ant response to potential herbivorous intruders (Oliveira et al., 1987; Vidal et al., 2016).

After placing a termite on the leaf, we quantified ant aggression over a 10-min observation period as the number of attacks (i.e., an ant lunged and bit the termite), the latency to the first attack, and the time before the termite was killed or fled from the leaf. We also quantified colony activity as the number of ants that entered the leaf during the observation period. An intruder test ended when the termite was killed or expelled from the leaf, or 10 min elapsed.

We carried out intruder tests on each control and sugar-supplemented tree at three time points during the treatment period: immediately before the sugar treatment began (Pre), 24 h into the treatment period (Post1), and 7 days after initial exposure (Post2). At each time point, we conducted intruder tests on two leaves per tree. Specifically, we used the fifth and sixth leaves from the top of the tree unless one was damaged, in which case we used the fourth leaf. We attempted to conduct intruder tests during consistent weather conditions. However, given the variable weather in the Amazon rainforest and our finite time at the field site, intruder tests were conducted during a range of light conditions, from overcast to full sun, and in light precipitation. We ensured an equal number of control and sugar-supplemented trees were tested during each weather condition to mitigate against any significant weather effects. Based on our observations, ant activity declined during heavy rain, so we did not conduct intruder tests during this weather condition. Heavy rain required we shift our behavior testing periods to include 24–48 h for Post1 at six sugar-supplemented and six control trees and between 5 and 7 days for Post2 at 11 sugar-supplemented and 13 control trees. All intruder tests were conducted between 0800 h and 1800 h.

2.4 | Statistical analysis

We tested for effects of supplemental sugar on (1) number of attacks, (2) likelihood of attacks, (3) colony activity (number of ants recruited), and (4) latency to first attack. For each response variable, we fit a generalized linear mixed-effect model using "lme4" version 1.1–33 (Bates et al., 2015) in R version 4.2.2 (R Core Team, 2022). Trial (Pre, Post1, Post2), Treatment (Control or Sugar-supplemented), and a Trial*Treatment interaction were included as fixed effects and ant colony ID was fit as a random effect. Pairwise contrasts were generated with "emmeans" version 1.8.6 (Lenth et al., 2019).

Whether termites were attacked or not was fit with a binomial distribution. The number of attacks, latency to first attack, and number of recruits (colony activity) were fit with a negative binomial distribution, which met required assumptions and was a better fit for over dispersion than the Poisson distribution (also confirmed via AIC and an "anova" from base R "stats" package; R Core Team, 2022). We used $\alpha = 0.05$. All model assumptions were checked using the "DHARMa" package (version 0.4.6; Hartig, 2022) and were met. R^2 values were calculated with the "MuMIn" version 1.47.4 (Bartoń, 2022). Plots were constructed using "ggplot2" version 3.5.1 (Wickham, 2016).

3 | RESULTS

The sugar-supplemented treatment significantly reduced the frequency of ant attacks on termite intruders (Figure 2) (Sugar-supplemented Pre-Post1: $B = -1.17$, $p < .001$; Sugar-supplemented Pre-Post2: $B = -.963$, $p < .001$; Control Pre-Post1: $B = -.161$, $p = .975$; Control Pre-Post2: $B = .072$, $p = .999$; model $R^2_m = .15$; model $R^2_{c=} .37$).

The sugar treatment also reduced the likelihood of ants attacking the termites (Figure 3; Sugar-supplemented Pre-Post1: $B = -2.65$, $p = .013$; Sugar-supplemented Pre-Post2: $B = -3.31$, $p = 0.001$; Control Pre-Post1: $B = -.641$, $p = .928$; Control Pre-Post2: $B = -.232$, $p = 0.999$; model marginal variance, $R^2_m = .15$; model conditional variance, $R^2_{c=} .37$).

The experimental treatment did not significantly affect colony activity (Figure 4). In both treatment groups, the number of ants recruited decreased in the first 24–48 h (Pre-Post1: $B = -.381$, $p = .029$) but not over the length of the 5–7 day experiment (Pre-Post2: $B = -.292$, $p = .123$; model $R^2_m = .03$; model $R^2_{c=} .23$).

There was no significant change in either treatment group in the latency to attack (Figure 5; Sugar-supplemented Pre-Post1: $B = .486$, $p = .317$;Sugar-supplemented Pre-Post2: $B = .231$, $p = 0.956$; Control Pre-Post1: $B = .394$, $p = 0.455$; Control Pre-Post2: $B = - .25$, $p = .887$; model $R^2_m = .07$; model $R^2_{c=} .29$).

4 | DISCUSSION

Our study aimed to provide insight into the proximate mechanisms maintaining an obligate ant-plant mutualism by testing the effect of providing the ants with an alternative sugar source. We found that providing *Pseudomyrmex dendroicus* colonies with a novel sugar source reduced the frequency with which they attacked and killed or expelled simulated herbivorous intruders (*Nasutitermes* sp. termites) on their *Triplaris americana* host plants. Over the same time period, no change in aggression toward herbivorous intruders occurred in control colonies. In regard to colony activity (i.e., number of patrolling ants), there was no detectable difference between control and sugar-supplemented groups. Thus, ant colonies with access to supplemental sugar remained on the host plant but were less likely to

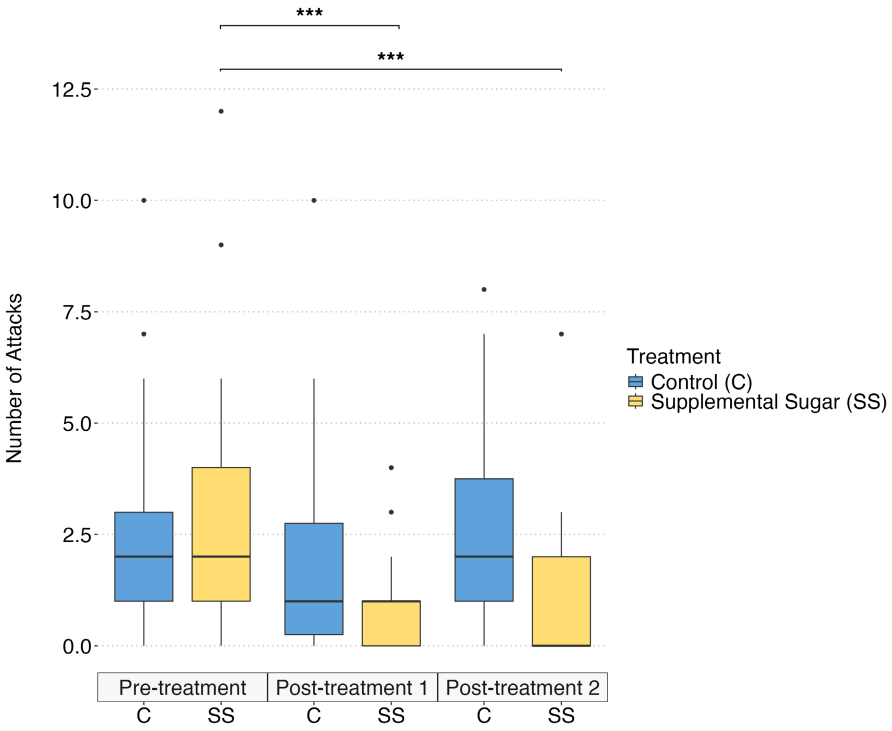


FIGURE 2 Number of attacks by *P. dendroicus* on the introduced termite intruders pre-treatment and post-treatment trials. Brackets with asterisks indicate statistical significance ($a = .05$) between trials for the sugar-supplemented treatment. The intruder tests were repeated over three trial periods: Immediately before the sugar treatment began (Pre-treatment), 24 h into the treatment period (Post-treatment 1), and 7 days after initial exposure (Post-treatment 2). The experiment included 17 control trees and 17 sugar-supplemented trees.

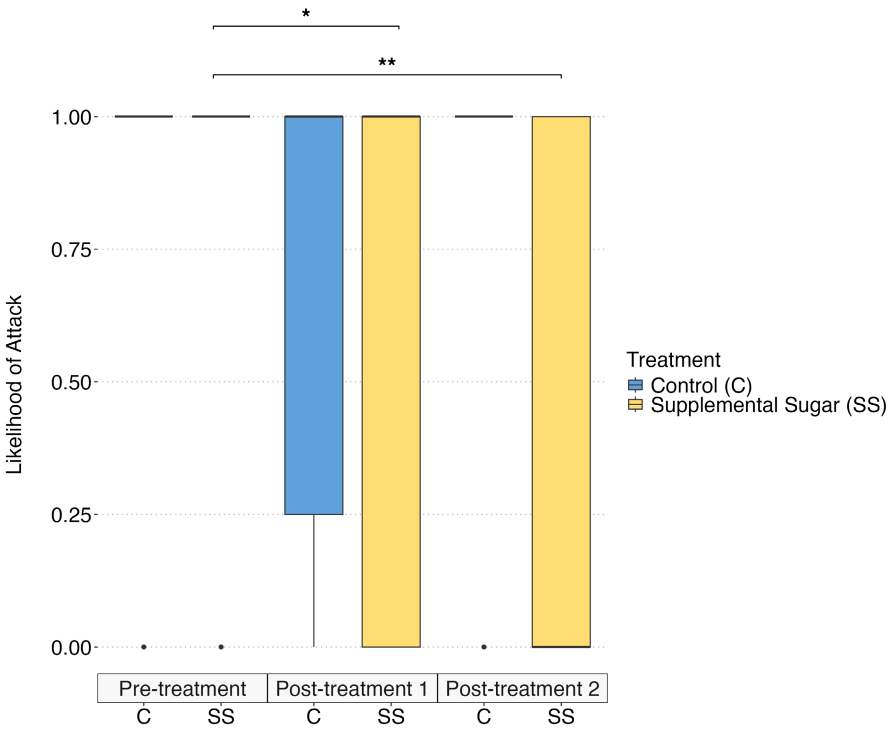


FIGURE 3 Likelihood of attack by *P. dendroicus* on the introduced termite intruders. Brackets with asterisks indicate statistical significance ($\alpha = .05$) between trials for the sugar-supplemented treatment. The intruder tests were repeated over three trial periods: Immediately before the sugar treatment began (Pre-treatment), after 24 h of sugar supplementation (Post-treatment 1), and after 7 days of sugar supplementation (Post-treatment 2). The experiment included 17 control trees and 17 sugar-supplemented trees.

attack herbivorous intruders. These findings are as predicted by the "attract and distract" hypothesis (Wäckers et al., 2017) but conflict with the "deficit", "fuel-for-foraging", and "predictable rewards" hypotheses, all of which predict that sugar supplementation should result in greater protection of the host plants by the ants.

One study that reports results similar to ours involves a mutualistic association between ants (Hymenoptera: *Formicidae*) and mealybugs (Hemiptera: *Pseudococcidae*) (Parrilli et al., 2021). Sucrose-rich baits attracted ants away from protecting mealybugs from predators and parasitoids, thereby weakening the mutualism (Parrilli et al., 2021). We used a glucose-rich sugar source (corn syrup), but observed similar changes in ant behavior. This suggests that changes in nutrient availability, regardless of sugar type, has the potential to disrupt ant mutualisms. However, in both our study and the study by Parrilli et al. (2021), the alternative sugar source was more concentrated than that provided by the host. The honeydew of coccoid scale

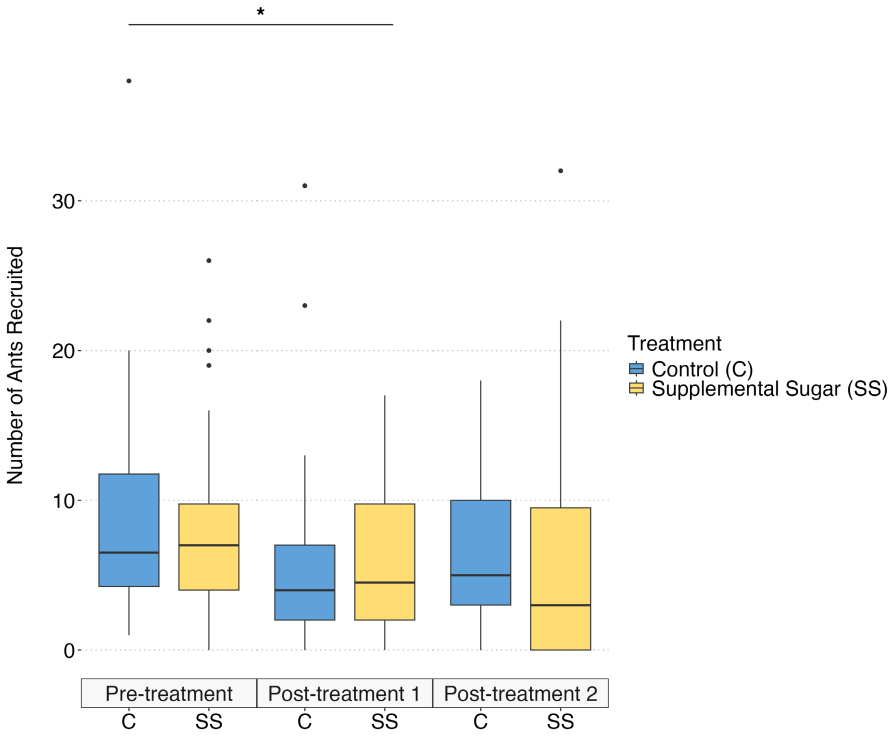


FIGURE 4 Ant colony activity. Activity was determined by the number of *P. dendroicus* that arrived on the leaf after the termite bait was placed. Brackets with asterisks indicate statistical significance ($\alpha = .05$) for a main effect between the pre-treatment and 24 h into the treatment period (post-treatment 1). The intruder experimental tests were repeated over three trial periods: Immediately before the sugar treatment began (Pre-treatment), after 24 h of sugar supplementation (Post-treatment 1), and after 7 days of sugar supplementation (Post-treatment 2). The experiment included 17 control trees and 17 sugar-supplemented trees.

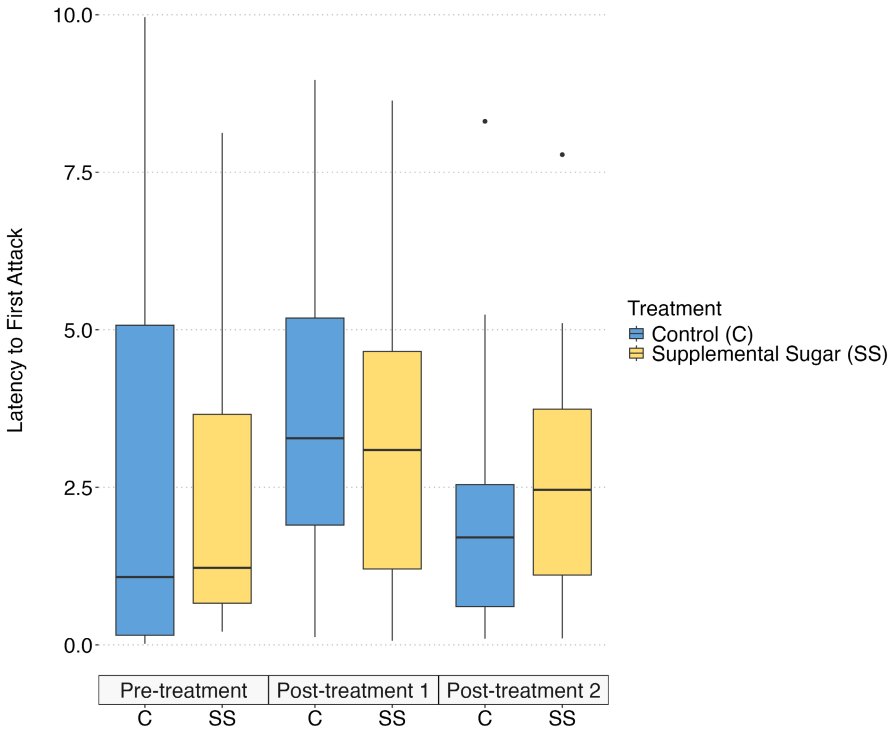


FIGURE 5 Latency to first attack by *P. dendroicus* on the introduced termite intruders pre-treatment and post-treatment trials. The intruder tests were repeated over three trial periods: Immediately before the sugar treatment began (Pre-treatment), after 24 h of sugar supplementation (Post-treatment 1), and after 7 days of sugar supplementation (Post-treatment 2). The experiment included 17 control trees and 17 sugar-supplemented trees.

insects varies between species but has been measured as between 9%–15% water and with small amounts of glucose (entirely absent for some species) (Ewart & Metcalf, 1956). Providing *P. dendroicus* with supplemental food with a lower sugar concentration could potentially produce different behavioral effects. Experiments varying the concentration and composition of supplemental sugar could clarify the likelihood of ant-plant mutualisms being disrupted by alternative sugar sources.

While shifting to more energy-rich food sources might be part of an adaptive foraging strategy, the resulting reduction in protection of the host plant could have cascading effects that destabilize the mutualism. Perhaps owing to its co-evolutionary history with *P. dendroicus*, *T. americana* has no other defenses against herbivory (de Melo Teles e Gomes, 2023). The duration of our study was insufficient to measure effects on the host plants, but other studies have shown that *T. americana* suffers in the absence of *P. dendroicus*. In one

ant removal experiment, the host plants experienced a fifteen-fold increase in leaf predation (Sanchez & Bellota, 2015). *P. dendroicus* is known as a highly selective and dependent ant symbiont, relying only on *T. americana* trees for food and shelter (González-Teuber & Heil, 2009; Sanchez, 2015). Thus, a weakening of the mutualistic relationship will likely adversely affect both species.

Beyond risking the ant colony's nest site, shifting to a new food source might also have direct negative effects on ant health by changing the balance of macronutrients in the diet (Mayer et al., 2014). Experimental manipulations of the macronutrient composition of ant diets has been shown to affect body composition, rates of reproduction, and lifespan (Feldhaar, 2014). For example, reductions in amino acids can reduce larval growth (Feldhaar, 2014). In a laboratory food choice experiment, Csata et al. (2020) found that Argentine ants (*Linepithema humile*) exhibited compensatory foraging responses to a wide range of nutrient deficiencies. Whether and how *P. dendroicus* colonies compensate for nutritional imbalances remains to be investigated. But in some other ant-plant systems, when the ants had an alternative sugar source they switched from tending honeydew-producing insects to consuming them, perhaps to compensate for amino acid deficiency (Gullan & Kosztarab, 1997; Offenberg, 2001).

Pringle et al. (2011) conducted laboratory experiments on a similarly highly-dependent ant-plant system in which the ants, *Azteca pittieri*, depend on their host plants (*Cordia alliodora*) for both food and shelter and protect the plants from herbivory. They found that when *A. pittieri* were provided with concentrated sugar baits, they were more, not less, aggressive toward simulated herbivores (Lepidoptera larvae). Whether the opposite results of their experiment and ours reflect behavioral differences between the ant species or differences in methodology remains to be determined. The concentrated sugar baits in Pringle et al.'s (2011) study were designed to mimic the composition (50% sucrose, 30% fructose, 20% glucose) and concentration (70%) of coccoid honeydew. By contrast, we essentially gave *P. dendroicus* access to pure glucose. Another potentially important difference between the two studies is the choice of herbivorous intruders. Caterpillars might pose a more serious threat to host plants than termites by directly injuring plant leaves, which triggers ant defense (Agrawal & Dubin-Thaler, 1999; Blatrix & Mayer, 2010). However, termites have been used in previous field experiments on *Pseudomymex-Triplaris* and other ant-plant mutualisms (Oliveira et al., 1987; Vidal et al., 2016), and based on our observations, the ants were just as likely to attack termites as they were to attack other insects that they encountered on their host plants leaves. Still, experiments with other types of herbivorous intruders and sugar sources will be required to establish whether the *Pseudomymex-Triplaris* mutualism is maintained by different proximate mechanisms than other ant-plant mutualisms and especially sensitive to disruption.

Our study highlights the cost–benefit balance that governs ant-plant mutualisms and documents how these relationships are potentially sensitive to disturbance and disruption. Mutualisms are considered to be one of the primary drivers of biological diversity (Bronstein, 2021). The disruption of one prevalent mutualism could potentially have cascading effects that destabilize an entire community. Further research is needed not only to clarify the mechanisms maintaining ant-plant mutualisms, but also to understand their role in ecosystem structure.

AUTHOR CONTRIBUTIONS

Sarah G. Hoffman and Luke Benson led conceptualization, data curation, investigation, methodology, writing of the original draft, and assisted in formal analysis. Conner S. Philson assisted in data curation, formal analysis, methodology, supervision, visualization, review and editing of the manuscript, and led formal analysis. Rachel Y. Chock assisted in methodology, review and editing of the manuscript, and led project administration and supervision. Joseph N. Curti assisted in methodology, supervision, project administration, visualization, and review and editing of the manuscript. César F. Flores-Negrón assisted in project administration and resources. Gregory F. Grether assisted in formal analysis, methodology, writing and editing of the manuscript, and led project administration and supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in OSF at http://www.doi.org/10.17605/OSF.IO/GKR2N.

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