Ants that defend plants receive sugar and protein

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The aggressiveness of ants in arid environments with scarce food supply helps protect plants against herbivorous arthropods. Credit: Laura Leal

Biologists Laura Carolina Leal and Felipe Passos have performed a series of experiments to determine how plants with extrafloral nectaries interact with ants in Brazil's Northeast region—specifically, in the interior of Bahia State, where the semiarid Caatinga biome predominates.

Extrafloral nectaries are nectar-secreting glands not involved in pollination, which provide carbohydrates to insects in exchange for defense against herbivores. The nectar attracts predatory insects that consume both the nectar and plant-eating arthropods, functioning as bodyguards.

"In contrast with the previous belief, we discovered that carbohydrate is only one of the forms of payment offered by plants to the ants that protect them. Another is protein, which ants obtain by consuming the herbivorous arthropods available on or around the plants they visit," said Leal, a professor in the Federal University of São Paulo's Institute of Environmental, Chemical and Pharmaceutical Sciences (ICAQF-UNIFESP) in Brazil.

"This finding contradicts the idea that payment is in sugar only," Leal told. "It shows that what ants gain from herbivores also matters. We discovered that ants may be more aggressive in environments where arthropods and other sources of protein are scarce, defending their food sources and hence protecting plants."

The study is published in *Biological Journal of the Linnean Society*. The research interests pursued by Leal and Passos focus on the various forms of insect-plant mutualism. "Mutualism is a form of interaction between two species in which each benefits from the interaction in some way. If it isn't advantageous for both species, but only for one, it's parasitism," Leal said.

"Several studies have shown that nectarivorous ants expel herbivores and enhance the reproductive success of plants with extrafloral nectaries. The greater the importance of extrafloral nectar to the ants, the better for the plants, as this increases the ants' aggressiveness toward herbivores. We decided to find out whether nectar is the only payment by plants for the ants' protection or whether eating herbivores might also be

advantageous to the ants."

Leal and Passos confirmed the hypothesis that plant attendance by more aggressive ants and the efficiency of their defense increase when the availability of carbohydrates and/or proteins to the ants is low, enhancing the relative value of both extrafloral nectaries and protein-rich herbivores to these insects.

The study was conducted on the campus of the University of Feira de Santana in Bahia. The region has a semiarid climate with an annual average temperature of 25.2 °C and rainfall averaging 848 mm per year. The vegetation in the Caatinga is xerophytic (adapted to life in a dry habitat), consisting of a mosaic of thorny shrubs and seasonally dry forests.

The researchers established 19 study plots measuring 16 square meters each, in early 2017. The plots were at least 30 meters apart, and mainly contained Turnera subulata, a clumping plant in the passionflower family known as white alder. This was the only plant with extrafloral nectaries in the plots. Its density varied from five to 218 individuals per plot.

"T. subulata has a pair of extrafloral nectaries on each petiole [the stalk that attaches the leaf blade to the stem] and inflorescence base," Leal said. The extrafloral nectaries are constantly visited by different ant species that can defend the plant against herbivores. The relative importance of any resource for animals is influenced not only by its abundance in the habitat but also by the number of individuals sharing it. Our first step was therefore to count the nests of ants that foraged in our study plots."

The researchers left five mixtures of carbohydrate and protein (sardine and honey) as bait in the soil of each plot between 7 and 11 a.m., when ants were most active at the site. One piece of bait was placed at the

center and the other four approximately three meters away at the corners.

"We waited until the ants located the bait and followed them back to their nests, even when these were located outside our study site," Leal said.

They counted the ant nests and estimated the abundance of protein and carbohydrate resources for ants in each plot. Because T. subulata is a prostrate herbaceous plant occurring in open habitats, it is visited mainly by soil-foraging ant species.

"We recorded 312 occurrences of 13 ant species on these plants. Most were visited by two or more ant species simultaneously," Leal said.

The most frequent species was Camponotus blandus (42 percent of occurrences), followed by Dorymyrmex piramicus (25.6 percent). Dead arthropods in the soil are the main source of protein for these ants.

The researchers used soil arthropod biomass as a proxy for protein availability to the ants that visited extrafloral nectaries in each plot. To obtain this metric, they installed five pitfall traps in each plot, again one at the center and one at each corner.

"The pitfall traps remained active for 24 hours. We filtered their content and dried it in an oven at 60 °C for 24 hours. The lower the average dry arthropod biomass collected from each plot was, the lower the local availability of protein to ants," Leal said.

Less protein, more aggressiveness

The researchers also observed the behavior of ants visiting extrafloral nectaries with regard to a simulated herbivore to determine whether the

availability of carbohydrate and/or protein in the habitat affected the efficiency of the ants' defense.

"We simulated the presence of herbivores on the plants using the larvae of Ulomoides dermestoides, a common predator of peanut seeds known as the peanut beetle or Chinese weevil. On the most apical branch of each focal plant, we placed one larva on the leaf that offered the best horizontal or near-horizontal platform for the insect. We allowed the larva to move freely on the leaf and waited for it to be found by the ants," Leal said.

The biologists identified the ants present on five plants in each plot and measured their efficiency in removing the simulated herbivores from the plants.

"When a larva was located, we observed the behavior of the ants with regard to the larva. We observed whether the larva was removed from the plant, whether the ants took the larva to the ground, pushed it off the plant, or consumed it where it was," Leal said.

According to her, the probability of interaction between the plants and more aggressive ant species was not influenced by the number of active extrafloral nectaries or the arthropod biomass found in the plots.

"However, the simulated herbivores were removed more frequently in plots with less arthropod biomass. This suggests that ants, regardless of species, become more aggressive toward other arthropods in protein-poor habitats. This increase in aggressiveness potentially increases the efficiency with which plants that have extrafloral nectaries are defended against herbivores," Leal said.

Unlike carbohydrates, protein resources are not renewable and are randomly distributed in the environment. Dead insects, for example,

have no predictable pattern of distribution and may be found almost anywhere. Once consumed, these dead insects are unavailable to other species of ants in the community.

"This led us to propose that plants with extrafloral nectaries may be more efficiently defended in protein-poor habitats regardless of how much they invest in interaction via nectar secretion," Leal said.

If so, even plants that secrete low-quality extrafloral nectar may be efficiently defended because the ants' behavior toward herbivores will be driven by their demand for protein and not for carbohydrates.

More information: Felipe C S Passos et al, Protein matters: ants remove herbivores more frequently from extrafloral nectary-bearing plants when habitats are protein poor, *Biological Journal of the Linnean Society* (2019). DOI: 10.1093/biolinnean/blz033

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