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SHORT COMMUNICATION

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Mechanical defenses of plant extrafloral nectaries against herbivory

Moshe Gish[a], Mark C. Mescher[b], and Consuelo M. De Moraes[b]

[a]Department of Biology, The Pennsylvania State University, University Park, PA, USA; [b]Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland

ABSTRACT

Extrafloral nectaries play an important role in plant defense against herbivores by providing nectar rewards that attract ants and other carnivorous insects. However, extrafloral nectaries can themselves be targets of herbivory, in addition to being exploited by nectar-robbing insects that do not provide defensive services. We recently found that the extrafloral nectaries of *Vicia faba* plants, as well as immediately adjacent tissues, exhibit high concentrations of chemical toxins, apparently as a defense against herbivory. Here we report that the nectary tissues of this plant also exhibit high levels of structural stiffness compared to surrounding tissues, likely due to cell wall lignification and the concentration of calcium oxalate crystals in nectary tissues, which may provide an additional deterrent to herbivore feeding on nectary tissues.

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Extrafloral nectaries are widespread in plants, having been reported in more than 100 families.[1-3] These nectar-secreting organs may be located on any above-ground plant part,[4] and function in plant defense against herbivory via the recruitment of ants and other predatory (and parasitoid) insects.[5] The protection that such insects provide in exchange for the nutritional reward offered by the plants is a classic example of plant-insect mutualism.[6] However, extrafloral nectaries can also be vulnerable to exploitation. For example, many invertebrates consume extrafloral nectar without providing protective services in return, including herbivores that feed on the plants they "rob"?[7-10] Furthermore, we recently reported that nectary tissues can also be subject to targeted feeding by insects, including species that are otherwise not primarily herbivorous.[11]

Opportunities for such exploitation are reduced by the "indirect" defense provided by defending mutualists. However, defending insects such as ants are not always present near the nectaries, nor are they effective against all plant visitors. Consequently, some plants might be expected to complement these indirect defenses by investing in the direct defense of extrafloral nectaries, which constitute valuable defensive organs. We recently found that the extrafloral nectaries of *Vicia faba*, as well as leaf tissues surrounding the nectaries, exhibit high levels of L-3,4-dihydroxyphenylalanine (L-DOPA), a non-protein amino acid toxic to insects. In some other plant species, embedding extrafloral nectaries in the tissues of other organs (e.g. pit nectaries[12]) may reduce their exposure to potential exploiters. We hypothesized that plants might also defend extrafloral nectaries by fortifying these structures with mechanical defenses that increase tissue stiffness. To explore this possibility, we assessed the stiffness of the extrafloral nectaries of *V. faba* plants compared to that of other regions of the stipules on which the nectaries are located.

Extrafloral nectary tissue was stiffer than tissue elsewhere on the stipule in all cases ($n = 20$ stipules examined). On average, nectary tissue was 2.28 ± 0.14 (SE) times stiffer than the rest of the stipule (paired-sample T-test, $t_{19} = 10.21$, $P < 0.0001$). This increased stiffness may be caused by the thickened and lignified walls of some of the cells in the extrafloral nectaries of *V. faba*.[13] Studies examining other plant species have also documented lignification of cell walls[14] and sometimes masses of sclereids[15] in extrafloral nectary tissues. The resulting increase in stiffness (and probably also toughness) may be expected to provide some degree of mechanical defense against herbivory, as previous work on foliar herbivory indicates that these characteristics adversely impact herbivore feeding performance.[16,17]

Extrafloral nectaries are also typically rich in calcium oxalate crystals, which are usually found in the parenchyma that lies underneath the secreting epidermis and sometimes create a mass at the core of the nectary.[18]

CONTACT Consuelo M. De Moraes consuelo.demoraes@usys.ethz.ch TH Zürich, Department of Environmental Systems Science, Biocommunication & Ecology, LFO G 18, Schmelzbergstrasse 9, 8092 Zürich, Switzerland.

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Concentration of calcium oxalate crystals in and around extrafloral nectaries has been extensively documented[14,19-25] and occurs in diverse plant taxa.[12,26,27] Calcium oxalate crystals in extrafloral nectaries may function as a sink for calcium ions that could otherwise interfere with the transport of sugars into the nectary;[28] however, they may also enhance the resistance of the nectary to feeding by insects with chewing mouthparts.[29,30] To efficiently extract the nutrients from plant tissue, most chewing insects must crush and disrupt cell walls in order to gain access to the cytoplasm.[31] Calcium oxalate crystals, which are harder than insect mandibles (>5 vs. 3 on Mohs scale, respectively[32,33]) and roughly the size of the surrounding cells, may reduce cell crushing efficiency by preventing mandible closure, similar to the probable main mode of action of silica phytoliths on insect mouthparts.[34]

Even when combined with chemical defenses, these mechanical characteristics do not provide total protection, as extrafloral nectaries can be damaged by insect feeding and are sometimes targeted for in preference to other plant tissues.[11] However, these mechanical defenses probably reduce vulnerability of the nectary to herbivory as well as its value as a food source. Moreover, such quantitative plant defenses (reducing the digestibility and/or palatability of the plant) are more immune to herbivore specialization than qualitative defenses (toxins), which may be metabolized and deactivated by coevolved herbivores.[35] Consequently, the mechanical characteristics of extrafloral nectaries, in combination with chemical defenses, may play an important role in the direct defense of critical plant organs that, in turn, mediate the indirect defense of other plant tissues.

Materials and methods

We measured the differences in stiffness between extrafloral nectaries of *V. faba* and the tissue that surrounds them. We mounted 20 stipules from 20, 18-day-old plants on adhesive tape that was attached (sticky side up) to the surface of a piece of flat Styrofoam. We did a penetrometer test to measure the initial modulus of rigidity of the spot being tested, using a texture analyzer (TA.XT2i, Stable Micro Systems, Surrey, UK) fitted with a 10° stainless steel cone moving at 0.5 mm/s. In each stipule, one measurement was done in the center of the extrafloral nectary and 3 measurements were done in random locations on the stipule. For each stipule, an average "stipule stiffness" was calculated from these 3 measurements and this value was compared with the measured stiffness of the extrafloral nectary on that stipule.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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