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Secretory activity of extrafloral nectaries shaping multitrophic ant-plant-herbivore interactions in an arid environment

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ABSTRACT

In this study, we evaluated the effects of the secretory activity of nectaries on multitrophic interactions involving ants and herbivores foraging on individuals of *Prosopis laevigata* (Fabaceae) located in the arid Tehuacan-Cuicatlán valley, Mexico. We observed that the secretory activity of nectaries of *P. laevigata* appeared to follow the predictions of the 'Optimal Defense Theory'. Specifically, our results suggest that due to high temperature and low humidity during the day, nectaries were more active at night, precisely when herbivores and ants were more abundant. Moreover, protective ants were always more abundant in the apical zones of the branches where youngest leaves are most susceptible to attack by herbivores. We hypothesized that *P. laevigata* could be optimizing nectar secretion in order to allocate defenses to maximize their individual fitness. Our study contributes to our understanding of how Optimal Defense Theory could shape multitrophic interactions between ants, plants, and herbivores mediated by secretory activity of nectaries in arid environments.

1. Introduction

Herbivore-plant interactions are among the most remarkable challenges for ecologists all around the world and have been shown to play central roles in the evolution of plant defense traits against herbivory (Coley and Barone, 1996; Rosenthal and Kotanen, 1994). A major and well-known defense mechanism against herbivory is the indirect and biotic defense generated by a third trophic level: herbivore enemies (Nahas et al., 2012; Rico-Gray and Oliveira, 2007). An example that occurs mainly in the tropics is mediated by the offer of extrafloral nectar (henceforth 'nectaries') to ants that protect their host plants against herbivores (Blüthgen et al., 2004; Lange and Del-Claro, 2014). Nectaries are distributed on different plant structures (e.g. spike, pedicel, bud, calyx, leaves, shoots, petioles, bracts, or stems) and occur in more than 108 families and 745 genera of ferns and angiosperms (Aguirre et al., 2013; Weber and Keeler, 2013). The secreted nectar is a highly nutritive resource, rich in sugar and amino acids (González-Teuber and Heil, 2009), which directly influences ant activity on plants (Blüthgen et al., 2004), and may increase ant colony fitness (Byk and Del-Claro, 2011).

Studies of mutualistic interactions between ants and extrafloral nectary-bearing plants have been carried out mainly in the Brazilian savanna (Cerrado) and some tropical dry forests (Del-Claro, 2004; Rico-Gray and Oliveira, 2007). Most of such studies have shown through ant-exclusion experiments that the absence of ants can increase herbivory, and reduce fruit set (Nascimento and Del-Claro, 2010) and seed production (Vesprini et al., 2003). However, despite widespread knowledge of the ecology of mutualistic outcomes of ant–plant interactions, mechanisms that regulate nectar secretion and shape ant-plant-herbivore interactions appear to be more complex and poorly understood (Heil, 2011; Villamil et al., 2013), particularly in less-studied environments such as deserts. Extrafloral nectaries are important bottom-up resources for many organisms in arid environments because of the low availability of sugar and water (Holland et al., 2010).

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Based on the histological and physiological complexity of extrafloral nectaries, the production and secretion of nectar might be highly costly, and its secretion may not be continuous (Heil et al., 2000). In general, the 'Optimal Defense Theory (ODT)' predicts that plant investment is directly proportional to the tissue value and the likelihood of it being successfully damaged by herbivores (McKey, 1974, 1979; Rhoades, 1979). For extrafloral nectary-bearing plants, Optimal Defense Theory also holds that plants should secrete more nectar on the most valuable organs (e.g. youngest leaves) and in periods when herbivore pressure is higher (Millán-Cañongo et al., 2014). Because nectar secretion is directly related to protection of ants against herbivores (Rico-Gray and Oliveira, 2007), different factors may influence the phenotypic plasticity of a plant species to optimize its trade-off between nectar secretion and defensive benefits (Holland et al., 2009). For example, in arid and semi-arid environments with high diurnal temperature and low humidity, many insects such as ants and herbivores are more active at night, mainly due to their ecophysiological limitations (Byk and Del-Claro, 2010; Dáttilo et al., 2014; Fagundes et al., 1996). Moreover, some studies have shown that herbivores have higher preference for young and more palatable leaves (Brunt et al., 2006; Coley and Kursar, 1996). Therefore, it is possible to expect that in these highly seasonal environments plants would secrete greater amounts of nectar at night when herbivore pressure is higher (Falcão et al., 2014; Rogers et al., 2003) and in more vulnerable tissues (e.g. apical branches) (Heil and McKey, 2003; Korndörfer and Del-Claro, 2006).

The aim of our study was to evaluate the effects of the secretory activity of extrafloral nectaries on multitrophic interactions involving ant-plant-herbivore association in a population of *Prosopis laevigata* (Fabaceae) in the arid Tehuacan-Cuicatlán valley, Mexico. We hypothesized that the number of ants foraging on plants would be higher near the meristems (apical zone of the branches), because this plant part may be the most susceptible to attack by herbivores. In addition, we postulated that due to milder weather conditions at night, there would be greater abundance of herbivores and ants. Specifically, we addressed the following questions: (i) Does the secretory activity of extrafloral nectaries of *P. laevigata* vary between day and night?, (ii), Are ants found more frequently foraging on the apical branches compared to more basal branches?; and (iii) Are ants and herbivores more abundant at night?

2. Material and methods

2.1. Study area

The study was done in an area close to the 'Helia Bravo Hollis Botanical Garden' (18° 20′ N and 97° 28′ W, elevation 1507 m), located in Zapotitlán de las Salinas, Puebla, Mexico. This area is in the floristic province of the Tehuacán-Cuicatlán valley, and is within the Mexican xerophytic region. Mean temperature is 18–22 °C, with a mean total annual precipitation of 400 mm. This region exhibits strong seasonality, with a long dry season, from November to May, and a green and short rainy season from June to October. The arid Tehuacan-Cuicatlan valley comprises ca. 10,000 km^2, and contains between 10 and 11.4% of the Mexican flora, of which 13.9% is represented by endemic species (365) (Dávila et al., 2002).

2.2. Species studied

The genus *Prosopis* contains approximately 44 species, distributed in arid and semiarid areas of America. *Prosopis laevigata* (H. B. ex Willd.) Johnst. M.C (Fabaceae: Mimosoideae) is an endemic and widely distributed species in central Mexico, and in some regions they are utilized for food, forage, fuel, construction material, and even medicine (Maldonado-Aguirre and de la Garza, 2000). *Prosopis laevigata* is a tree reaching up to 8 m tall and commonly found in arid and semi-arid regions of Mexico (Fig. 1A). It has a broad, densely branched canopy that creates a shady and cool environment, which allows the establishment of many less light-tolerant plant species. This species has axillary spines paired (4–7 mm long) on trunks and branches. Leaves (7–13 cm long) are compound and consist of 1–2 pairs of primary leaflets divided into 18–30 pair secondary leaves (Maldonado-Aguirre and de la Garza, 2000). Moreover, the leaves of *P. laevigata* do not exhibit a reddish coloration at any time during their ontogeny, indicating that biotic defense exerted by ants may be an important mechanism of defense against herbivory. *Prosopis laevigata* also possesses cup-shaped elevated nectaries on the petiole and rachis of the leaves (Fig. 1), which attract different soil dwelling ant species (Vilela and Palacios, 1997).

2.3. Data collection and statistical analysis

Sampling was conducted at the beginning of April 2014 (the driest time of the year) in an area of ca. 1000 m^2. Note that, *P. laevigata* is not spatially abundant in our study area, and in order to reduce any bias related to plant ontogeny and soil type, we selected only 16 trees of *P. laevigata* separated by distances of 10–30 m. Specifically, trees were selected based on the following attributes: i) the same vegetation type, mesquite woodlands, ii) similar size (<20 cm diameter at breast height), and branching pattern; iii) tree heights ranging from 1.50 to 2.50 m in order to be accessible to the collector.

To evaluate the patterns of nectary activity during day and night, we randomly isolated, in each tree, 10 apical nectaries in five branches using a mesh bag and a non-toxic resin (Tanglefoot®) around the base of branches, we also removed other vegetation that may be a bridge for ants. To avoid nectar consumption by nectary-visitors, and consequently, a decrease in the amount of nectar available, we prevented the access to the nectaries by winged and non-winged visitors 4 h per day (0700–1100 h AM) and 4 h over night (1900–2300 h PM) prior to the beginning of the measurements. Based on previous observations, this time period was considered enough for nectar accumulation and to prevent evaporation. Size of extrafloral nectaries was very small (Mean ± SD: 0.71 ± 0.12 mm) (Fig. 1) and nectar secretion was extremely low, which prevented us from measuring nectar volume (μL), even using different shape or size of microcapillaries. However, it was possible to clearly see the activity of nectaries based on turgor and nectar accumulation on these glands. Thus, we counted how many nectaries per plant were inactive (Fig. 1B) or active (Fig. 1C). We then calculated the percentage of active nectaries per plant in both day and night periods.

To evaluate the patterns of ant-plant-herbivore interactions, we established in each plant the following treatments: (i) Abundance of ants foraging on the apical and basal branches of *P. laevigata* - we categorized apical branches as those inserted in the first 20 cm (from apex to stem), and the basal branches were all other parts of the plants. Two observers simultaneously counted the number of ant workers per tree on apical and basal branches during 5 min and in five branches per tree. We observed that only a few ants changed category (apical and basal), and in those cases a single ant could be counted twice. However, most ants were clearly separated between the two categories. All ants recorded were of the same species, and therefore, there was no difference in the use of basal or apical branches by different ant species. This procedure was done during the day (between 1000 and 1400 h) and night (between 2000 h and midnight). (ii) Abundance of herbivores foraging on individuals of