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Short communication

Insect visitation of peduncular and petiolar extrafloral nectar glands on castor bean (*Ricinus communis* L.) plants in Southern California

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Castor bean (*Ricinus communis* L.) is a myrmecophytic plant species with specialized extrafloral nectar (EFN) glands that serve to attract predatory insects, which in return defend plant-tissues against herbivores. The EFN glands on castor bean plants are located along the leaf petioles as well as on the peduncles of its imperfect (unisexual) flowers. This field-project evaluates the richness, diversity, and species assemblage of insects visiting EFN glands located on (female and male) flower peduncles and leaf petioles on castor bean plants growing in a Southern California coastal landscape. We detected that EFN glands on female-flower peduncles were visited by an insect community that was distinct from that of the other two EFN gland types on castor bean. Additionally, the insects visiting EFN glands on male-flower peduncles more closely resembled those observed visiting EFN glands on leaf petioles. We conclude that the observed differences in the biotic defense of foliar and unisexual floral tissues on castor bean are congruent with the optimal plant-defense strategy of a monoecious pioneer species.

Key words: Ant-plant interactions, monoecious, mutualism, myrmecophyte, plant-defense optimality, sexual dimorphism.

INTRODUCTION

Extrafloral nectar (EFN) glands are specialized plant structures that secrete nectar but are neither part of the flower structure nor associated with pollination (Rico-Gray and Oliveira, 2007). EFN glands are visited by a large variety of predatory insects, such as ants, wasps and parasitoids (Del-Claro et al., 2013), which often serves to increase both the predation and parasitism rates of herbivorous insects feeding on plants bearing EFN glands (Gentry, 2002). However, the primary literature advances at least four hypotheses regarding the evolutionary role of EFN glands: (1) they help plants excrete excess carbohydrates (Beattie, 1985); (2) they attract predatory insects to defend plant tissues from herbivores (Byk and Del-Claro, 2011); (3) they deter ants from interrupting or interfering with pollination (LeVan et al., 2013); and (4) they discourage ants from tending phloem-feeding insects, such as Mealybugs (Becerra, 1989).

Castor bean (*Ricinus communis* L.) plants are a non-native species component of many habitats in southern

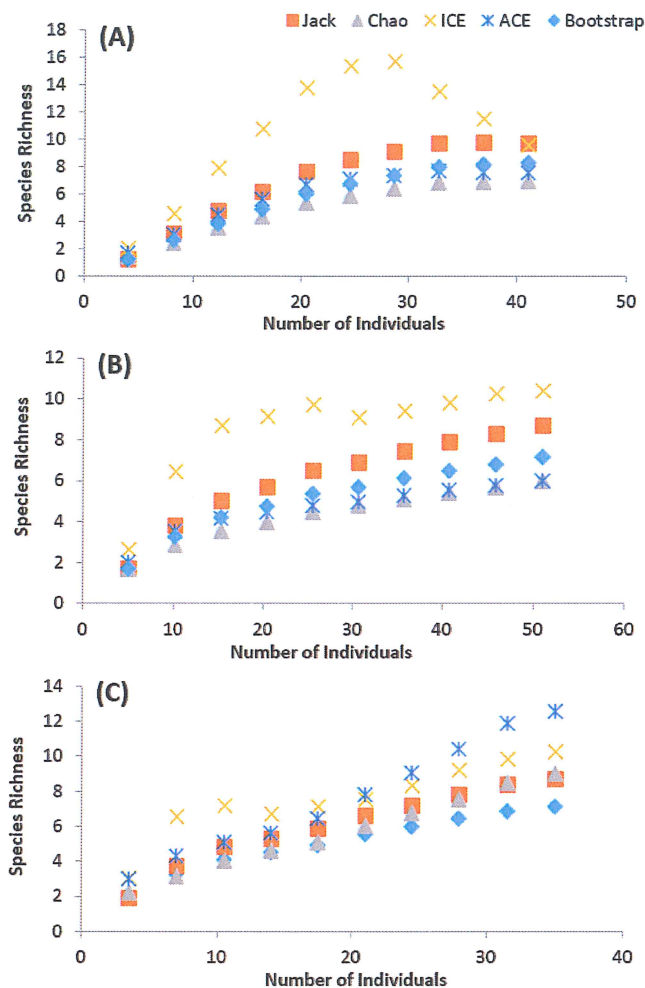*Corresponding author. E-mail: vcarmona@lmu.edu. Tel: +13103381968.

Figure 1. Species richness estimates of insects visiting castor bean EFN glands located on (A) female-flower peduncles, (B) male-flower peduncles, and (C) leaf petioles.

California, and have EFN glands located along its leaf petioles as well as on the peduncles of its imperfect flowers. Additionally, castor bean flowers do not produce nectar rewards (Rizzardo et al., 2012). As such, castor bean plants provide an opportunity to evaluate the role of EFN glands in biotic plant-defense via intraspecific contrasts of the insects attracted to different gland types. The objective of this project was to evaluate the community structure of insects visiting the EFN glands located on female- and male-flower peduncles as well as leaf petioles of castor bean plants in a southern California coastal landscape. We hypothesized that the species richness, diversity and composition of insects visiting castor bean EFN glands would diverge with respect to gland type due to differences in the optimal defense of foliar and unisexual floral tissues in a fast-growing (r-

selected) pioneer species.

MATERIALS AND METHODS

We selected 10 flowering castor bean plants growing on a coastal sage scrub habitat north of Loyola Marymount University that is adjacent to Ballona wetlands in Los Angeles, California during November 2013. The castor bean plant is a monoecious species with unisexual flowers that are grouped along an inflorescence. The morphospecies and relative abundance of insect visitors were recorded for 30 min at EFN glands located on peduncles of both female-flowers and male-flowers, as well as on leaf petioles.

Estimates of species richness were calculated based on field data-patterns of insect visitations to each of the three EFN gland types (female-peduncular, male-peduncular and petiolar) using the following multivariate predictors in the software EstimateS (Colwell, 2006): Chao I, ICE, ACE, Jackknife I, and Bootstrap. Shannon-Weiner diversity indices of insect morphospecies visiting the three different EFN gland types were evaluated using a rarefied sub-sampling technique in EstimateS (Colwell, 2006), which generates rarefaction-curves for the diversity index of each gland type that then allows for comparisons based on an equal number of individuals (Gotelli and Colwell, 2001). Evenness of insect morphospecies visiting each of the three EFN gland types was evaluated by means of rank-abundance curves, wherein a slope of zero represents the highest value of species evenness (unity). Species evenness is a measure of equitability among the relative abundances of all the represented species in a given sample and is inversely proportional to species dominance, wherein most individuals in a given sample are represented by only a few species (Mulder et al., 2004). Differences in distribution of taxonomic orders represented by insect visitors across the three EFN gland types were evaluated using a 2-factor (contingency table) Chi-square test.

RESULTS

We observed a total of seven morphospecies of insects (n=41 individuals) visiting female-peduncular EFN glands, 6 morphospecies (n=51 individuals) visiting male-peduncular EFN glands, and 6 morphospecies (n=35 individuals) visiting petiolar EFN glands. Based on multivariate fitting, the estimated species richness of insect visitors to female-peduncular EFN glands ranged between 7-10 species (Figure 1A), visitors to male-peduncular EFN glands ranged between 6-10 species (Figure 1B), and visitors to petiolar EFN glands ranged between 7-13 species (Figure 1C). Rarefaction curves show that, based on n=35 insect visitors, diversity was greatest at female-peduncular EFN glands (H=1.61) and petiolar EFN glands (H=1.20) than at male-peduncular EFN glands (H=1.00) (Figure 2). Rank-abundance curves show that female-peduncular EFN glands had the greatest species evenness of insect visitors, while both male-peduncular EFN glands and petiolar EFN glands showed similar patterns of species evenness (Figure 3). The distribution of taxonomic orders of insect visitors differed significantly among the three different EFN gland types (x^2=2452.162, df=4, p<0.001) (Figure 4A-C). Hymenopteran visitations were most frequent at both
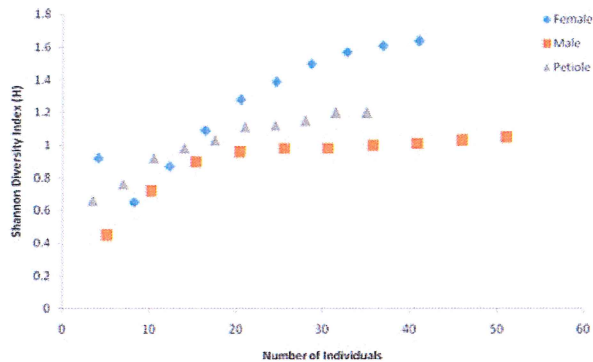
Figure 2. Morphospecies diversity of insects visiting castor bean EFN glands located on female-flower peduncles, male-flower peduncles, and leaf petioles.
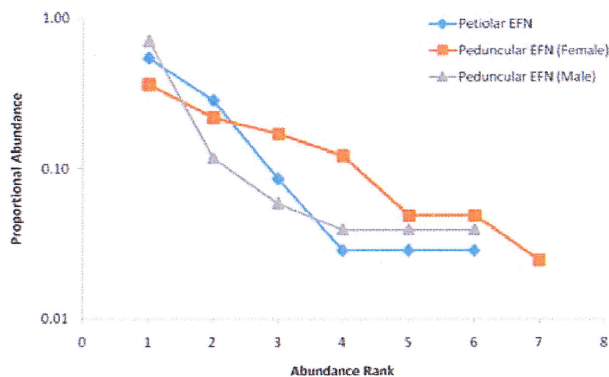

Figure 3. Rank-abundance curves for insect morphospecies visiting castor bean EFN glands located on female-flower peduncles, male-flower peduncles, and leaf petioles.
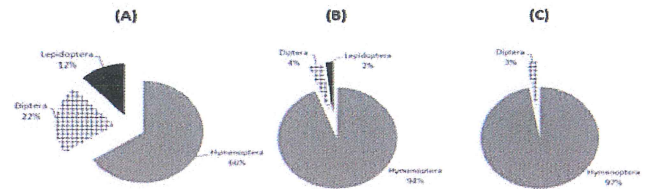

Figure 4. Taxonomic order of insects visiting EFN glands located along (A) female-flower peduncles, (B) male-flower peduncles and (C) leaf petioles of castor bean plants growing in a Southern California coastal landscape.

male-peduncular EFN glands (94%) and petiolar EFN glands (97%). Dipteran visitations were most frequent at female-peduncular EFN glands (22%). Lepidopteran visitations were observed only at female-peduncular (12%) and male-peduncular (2%) EFN glands, but were never observed at petiolar EFN glands.

DISCUSSION

Intraspecific comparisons of the insect communities visiting castor bean EFN glands located on female-flower peduncles, male-flower peduncles, and leaf petioles, showed marked differences in terms of species richness, diversity and composition. Multivariate estimates indicate that we detected between 70-100% of the insect species visiting both female-peduncular and male-peduncular EFN glands, as well as 46-86% of the insect species

visiting petiolar EFN glands. The highest species diversity of insects was attracted by female-petiolar EFN glands, while the diversity of insects visiting male-petiolar EFN glands was more comparable to that of the low diversity of insects visiting petiolar EFN glands. Based on the relative slopes of each rank-abundance curve, the species evenness was also highest among insects visiting female-petiolar EFN glands. Both male-peduncular EFN glands and petiolar EFN glands showed a similar lower species evenness, indicating that the majority of insect visiting these EFN glands were dominated by a few species.

Hymenoptera was the most frequent insect order observed visiting all three EFN gland types on castor bean plants, but represented the overwhelming majority (>94%) of insect visits to male-peduncular glands and petiolar glands. However, this pattern varied with species identity and EFN gland type. Honey bees (Hymenoptera: *Apis mellifera* L.) accounted for 71% of the total insect visits to male-peduncular EFN glands and 29% of the visits to petiolar EFG glands, while accounting for 37% of the visits to female-peduncular EFN glands. Honey bees are important in increasing seed yields of the castor bean plant due to its role in pollination (Navatha and Sreedevi, 2012; Rizzardo et al., 2012). However, there is evidence that the castor bean plays an antagonistic role in the relationship, as the pollen from this plant significantly decreases the lifespan of honey bees (de Assis Junior et al., 2011). Insects in the order Diptera were also observed at all three EFN gland types, but visits to female-peduncular EFN glands were almost an order of magnitude higher in terms of frequency to those observed visiting both male-peduncular EFN glands and petiolar EFN glands. While several Diptera species have been documented to pollinate castor bean plants (Mitra, 2010; Navatha and Sreedevi, 2012), herein we further showed a preference for the visitation of female-peduncular EFN glands. Lepidoptera was the only insect order observed to exclusively visit peduncular EFN glands. However, visitations by Lepidoptera to female-peduncular EFN glands were approximately an order of magnitude higher in terms of frequency to visits of male-

peduncular EFN glands.

In terms of the evolutionary role of EFN glands in the attraction of predatory insects (Baker-Méio and Marquis, 2012; Del-Claro et al., 2013), our study shows that biotic-defense strategies can vary intraspecifically with EFN gland type (Bluthgen et al., 2007; Bronstein, 2009). More specifically, female-peduncular EFN glands on castor bean plants are visited by a distinct assemblage of insect species relative to adjacent male-peduncular EFN glands, which instead more closely resembled the species assemblage of insects visiting petiolar EFN glands. The plant-defense strategy observed in castor bean is in line with that of a fast-growing (r-selected) monoecious species with unisexual flowers; wherein female-flowers are key to seed production, male-flowers senesce shortly after pollen release (personal observation), and leaf-tissues are short-lived (Coley et al., 1985). However, the number of EFN glands (Goss et al., 2011) and the quality of EFN gland secretions (Carmona-Galindo et al., 2014) may also play an important role in characterizing the variation of biotic-defense optimization in myrmecophytic species (Thompson, 2005).

While studies of plants with unisexual flowers have focused on pollinator behavior (Delph, 1996; Kawagoe and Suzuki, 2002), sexually dimorphic myrmecophytes offer an opportunity to highlight insect behavior with respect to extrafloral rewards on both reproductive and non-reproductive plant tissues (Rose et al., 2013). As such, future studies on sexually dimorphic myrmecophytes should focus on the intraspecific contrasts of reward-chemistry, such as the chemical composition of EFN secretions and proteinaceous food bodies.

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