ENVIRONMENTAL EFFECTS ON NECTAR PRODUCTION AND POLLINATOR INTERACTIONS WITH THE FLOWERING PLANT, *RUELLIA NUDIFLORA*

ALLISON M. CITRO, SAMUEL J. HAYNOR, BRIGETTE A. JONES
SONIA LEI, AND HA LINH VU

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Abstract. Plant-pollinator interactions can vary across environmental gradients. *Ruellia nudiflora*, a wild petunia, is an early successional plant with flowers visited by several bird and insect pollinators. We examined nectar production and consumption per flower in a shadier site versus a sunnier site. We did not find a difference in per flower nectar production or consumption between the two sites. Plant-pollinator interactions may not vary at the level of individual flowers across environmental gradients in this species, but instead may vary at a larger scale.

Keywords: nectar, pollinator preference, neotropical flower

INTRODUCTION

Flowers and the nectar they produce are valuable resources for many flowering plants and their nectar-consuming pollinators. As a result, plants invest heavily in floral display to attract pollinators (Galen 2000) and expend as much as 37% of their daily photosynthetic energy in nectar production (Southwick 1984). Nectivorous pollinators, such as hummingbirds, spend much of their day foraging and require daily nectar volumes far above their body weight (Powers and Conley 1992). Although the nature of plant-pollinator interactions is well studied (reviewed in Proctor *et al.* 1996), the effect of environmental variation on these relationships is less well understood.

Our study focused on per-flower nectar production and consumption in two sites to understand how the rewards offered by a flowering species differed across environments. We chose *Ruellia nudiflora* (Acanthaceae), a wild petunia with a large range across the Americas. *Ruellia nudiflora* is a ubiquitous, opportunistic plant that can grow across a variety of soil moisture, sunlight, and temperature conditions. We compared nectar production and consumption per flower between two sites that received different amounts of sunlight in the morning.

The tropical dry forest habitat at Palo Verde National Park provided an ideal site to examine the effects of sunlight on both nectar production and consumption. Some

neotropical plants are known to have low intraspecific variation in per flower nectar production (McDade and Weeks 2004). In addition there is a preference for warmer nectar by some pollinators due to the decreased energetic cost of warming this nectar to their body temperature (Lotz *et al.* 2003, Dyer *et al.* 2006). We hypothesized that in different environmental conditions, per flower nectar production in conspecifics would remain constant while pollinator nectar consumption would vary. We predicted that (1) the per flower nectar production in *R. nudiflora* would be the same at both sites and (2) nectar consumption would be lower at the shadier site, assuming shadier sites would have lower nectar temperatures.

In addition to evaluating plant-pollinator interactions through per flower nectar production and consumption between sites, we also considered the interactions at the level of the plant. The floral display of a plant is a prominent visual cue for pollinators and might be more influential in attracting pollinators than the actual amount of nectar produced by a flower. In addition, more flowers per plant might indicate more nectar per plant, if flowers contain similar quantities of nectar. We predicted that the sunnier site would have a lower number of flowers per plant due to lower soil moisture and extensive cattle disturbance. Because of potential lower number of flowers per plant, we also predicted that the sunnier site would have less total nectar per plant.

METHODS

We chose two patches of *R. nudiflora* with different abiotic conditions on 10 January, 2007 at Palo Verde Biological Research Station, Costa Rica. The first site was in direct sun throughout the day and had drier soil that was highly disturbed. Our second site was damper, received moderate sun, and had less disturbed soil.

To collect and measure nectar, we extracted nectar from each flower with 10 μL capillary tubes. Nectar volume per flower was calculated by using the ratio of the length of the capillary tube to the measured length of nectar: [(nectar length / capillary tube length)* 10 μL] = μL of nectar per flower.

To determine the amount of nectar produced per flower throughout the morning, we haphazardly bagged two flowers from each of ten plants at both sites at 0700 and extracted the nectar at 1100. We averaged the amount of nectar from two flowers on each of the ten bagged plants at both sites, and we counted the total number of flowers open on each of these plants. At 1100, we measured the nectar standing crop of one flower from

each of forty un-bagged plants. We assumed the difference in nectar between the bagged and un-bagged flowers at each site to be due to pollinator nectar consumption and not due to other factors, such as evaporation or re-absorption of the nectar. Using flowers per plant and nectar production per flower, we calculated the approximate nectar available per plant for each *R. nudiflora* examined.

We compared nectar production and pollinator nectar consumption (square-root transformed) between sites using a one-way ANOVA followed by a Tukey Highly Significant Difference (HSD) test (α=0.05). Based on our *a priori* hypothesis that the sunnier site would have a lower number of flowers per plant, we performed a one-tailed t-test assuming unequal variances. To test our *a priori* hypothesis that the sunnier site would have lower nectar production per plant, we conducted a one-tailed t-test. Due to low sample sizes for nectar production per plant, we also conducted a power analysis to determine how many samples we would have needed to determine a significant difference in nectar production per plant between the two sites.

RESULTS

We found an overall difference in nectar measurements between the four treatments ($F_{3,178}$=15.52, P < 0.001, Fig. 1). Specifically, we did not find a difference in nectar production between the sunnier and shadier site. We found a significant difference in morning pollinator nectar consumption between the two sites. At the sunnier site, we found that bagged flowers had 27% more nectar than the un-bagged flowers. At the shadier site, bagged flowers had 58% more nectar than the un-bagged flowers.

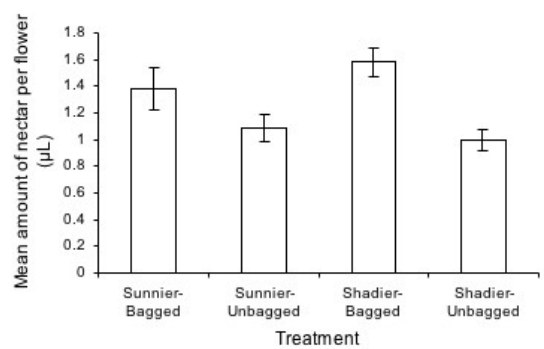

Figure 1. Mean amount of nectar in bagged (B) and un-bagged (U) flowers at the sunnier (Su) and the shadier (S) site with error bars representing ± SE. The amount of nectar represents nectar production throughout the morning for four hours in the bagged flowers and the standing nectar crop in the unbagged flowers.

The sunnier site had 300% more flowers per plant than the shadier site (t_{15}=2.28, P=0.04; Fig. 2). We found a marginally significant difference in approximate nectar production per plant between the sunnier site (mean ± SE=18.06 ± 5.87 µL) and the shadier site (mean ± SE=6.18 ± 2.29 µL) (t_{15}=1.97, P=0.07). We determined that a sample size of

71 plants would have given a statistically significant difference in approximate nectar production per plant between the two sites.

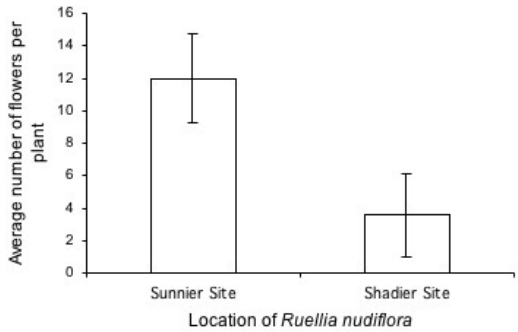

Figure 2. Mean number of flowers per plant between the two sites with error bars representing ± SE. Number of plants sampled for the sunnier and shadier site are N = 9 plants and N = 8 plants, respectively.

DISCUSSION

In our exploration of production and consumption of *R. nudiflora* nectar, we did not find variation in nectar production but did find differences in nectar consumption between the sunnier and shadier sites. Our first prediction, that nectar production would not vary between the sites, was supported. The similarity in per-flower nectar production between the two sites supports a previous study showing that environmental variables are not important in determining per-flower nectar production in neotropical plants (McDade and Weeks 2004).

Our second prediction, that the shadier site would have lower per flower nectar consumption, was also supported. Although we predicted nectar consumption would be higher at the sunnier site because the nectar would be warmer and therefore more attractive to pollinators, we did not test the temperature of the nectar between the two sites. In the hot Palo Verde environment, temperature may not vary greatly between sunlit and shaded site. Instead, it is possible that there is preferential selection by pollinators for certain environments for reasons other than that which we examined.

We found that there were more flowers per plant in the sunnier site than in the shadier one, refuting our third prediction that the sunnier site would have a lower number of flowers per plant, potentially due to lower soil moisture and extensive cattle disturbance. Soil moisture and disturbance might not be the determining factor in per plant flower production. The number of flowers per plant may be more biologically significant than per flower nectar production in determining the reproductive success of a plant because floral display may be more important for pollinator attraction in this system. While nectar per flower is important in encouraging pollinator revisitation, floral display may be more important in attracting pollinators initially. The higher nectar consumption at the sunnier site may have been driven by

pollinator preference for the site's large and attractive floral display.

We found a marginally significant difference in mean nectar production per plant between the two sites, a trend driven by the higher number of flowers per plant at the sunnier site. The floral display of a plant is influential in attracting pollinators, and pollinators will likely visit these plants and seek out the nectar rewards within each flower. However, plants with higher total nectar production may be able to serve a higher number of pollinators; therefore, there may be an increase in the plant's reproductive success at sunnier sites. Future studies might explore how total nectar production and reproduction per plant varies with experimental changes in environmental conditions.

Many other factors besides sunlight could have contributed to the differences in abiotic conditions that contribute to nectar production and consumption between the two sites. For example, one attribute we failed to explore was the temperature of the nectar at the two sites, which may have given more information about pollinator preference. Nectar temperature, however, may not be an important factor in pollinator preference in neotropical dry forests, as it is in temperate forests, because the ambient temperature is high in both shaded and sunny tropical sites. Future studies exploring plant-pollinator interactions should integrate pollinator preference across a variety of scales, from floral traits and rewards to environmental conditions.

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