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# VISITATION OF HELIOTROPE AND WESTERN PURSLANE FLOWERS BY *HESPEROPSIS GRACIELAE* (LEPIDOPTERA: HESPERIIDAE)

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## ABSTRACT

We conducted 2 studies on nectar-feeding by MacNeill's sootywing, *Hesperopsis graciellae* (MacNeill), a rare skipper, near the lower Colorado River in Cibola Valley, Arizona. In the first study, we examined rates of visitation by adults to *Heliotropium curassavicum* (Boraginaceae) inflorescences and *Sesuvium verrucosum* (Aizoaceae) flowers on potted plants. Visitation rates per plant or per inflorescence or flower did not differ between species when species were placed alone or together or when plants were placed in sun or shade. Frequencies of landings on both species were greater on plants in sun than shade, suggesting sooty-wings visually respond to flowers when foraging for nectar. In the second study, we measured masses of sugar in *H. curassavicum* inflorescences on wild plants after visitation by male or female adults. Inflorescences visited by females contained more sugar, but similar numbers of flowers, than those visited by males. Amounts of sugar remaining in inflorescences visited by females, but not males, decreased as visitation-times increased. Ingestion of nectar was apparent only in females. Both plant species can provide *H. graciellae* adults with nectar in conserved or created habitat.

Key Words: skippers, *Heliotropium curassavicum*, *Sesuvium verrucosum*, flower visitation

## RESUMEN

Realizamos 2 estudios sobre la alimentación de néctar de una mariposa poco común, *Hesperopsis graciellae* (MacNeill), cerca de la parte baja del Río Colorado en el Valle Cibola en Arizona. En el primer estudio, examinamos la tasa de visitación de los adultos sobre la inflorescencia de *Heliotropium curassavicum* (Boraginaceae) y flores de *Sesuvium verrucosum* (Aizoaceae) sembradas en macetas. La tasa de visitación por planta o por inflorescencia o flor no fue diferente entre las especies cuando las especies fueron puestas solas o juntas o cuando las plantas fueron puestas en el sol o la sombra. La frecuencia de aterrizaje de ambas especies fue mayor sobre plantas puestas en el sol que en la sombra, esto sugiere que las mariposas responden visualmente a las flores cuando están buscando néctar. En el segundo estudio, medimos masas de azúcar en inflorescencias de *H. curassavicum* en plantas silvestres después de la visitación de machos y hembras adultos. Las inflorescencias visitadas por hembras tenían mas azúcar, pero un número similar de flores, que las visitadas por machos. La cantidad de azúcar que quedó en las inflorescencias visitadas por hembras, pero no por los machos, disminuyó con el aumento del número de visitas. La ingestión de néctar fue aparente solamente en las hembras. Ambas especies de plantas pueden proveer los adultos de *H. graciellae* con néctar en hábitats conservados o criados.

MacNeill's sootywing, *Hesperopsis graciellae* (MacNeill) (= *Pholisora graciellae* MacNeill), is a small (wingspread 18-24 mm, MacNeill 1970) dark-brown skipper (Lepidoptera: HesperIIDae). It is found along the Colorado River downstream of the Grand Canyon and along the river's tributaries in Utah, Nevada, California, and Arizona (Emmel & Emmel 1973; Austin & Austin 1980; Stanford 1980; Nelson & Andersen 1999). Due to the sootywing's rarity, it has been granted the U.S. state conservation ranks (Master 1991) of S1 (critically imperiled) in Nevada and S2 (imperiled) or S3 (rare or uncommon but not imperiled) in California and Arizona. Larvae of *H. graciellae* develop only on leaves of the

wind-pollinated shrub *Atriplex lentiformis* (Torrey) S. Watson (Chenopodiaceae) (MacNeill 1970), and adults prefer to oviposit on host plants with high water and nitrogen contents (Wiesenborn & Pratt 2008). Adults fly during Apr through Sep (Pratt & Wiesenborn 2009). Sootywings appear to require shade to thermoregulate in their hot, sunny environment. They fly more frequently within plant canopies than in the open (MacNeill 1970) and have a comparatively-low tolerance of exposure to direct sunlight (Wiesenborn 1999).

Lack of nectar on *A. lentiformis* forces *H. graciellae* adults to seek nectar from non-host plants. Availability of non-host plants providing

adults with nectar has been found to influence the larval distribution of a monophagous butterfly (Murphy 1983). Two nectar-producing plants sympatric with the sootywing along the lower Colorado River Valley are heliotrope, *Heliotropium curassavicum* L. (Boraginaceae), and western purslane, *Sesuvium verrucosum* Rafinesque (Aizoaceae). *Heliotropium curassavicum* is a perennial that sends up scattered stems (<0.5 m high) from underground rootstocks (Munz 1974). Flowers occur in inflorescences of 2-4 (Jepson 1993) scorpioid cymes and contain 5, partially-fused petals that form a star-shaped, 3-6 mm-broad white corolla (Munz 1974). The yellow centers of heliotrope flowers usually turn purple as flowers sequentially mature on each cyme (Munz 1974). *Sesuvium verrucosum* is an uncommon (Jepson 1993), native perennial that forms a spreading mat or small (<0.5 m high) shrub with fleshy stems and leaves (Munz 1974). Flowers are solitary and composed of five, 1-cm long sepals that form a star-shaped calyx colored pinkish-purple inside (Munz 1974).

Conservation or creation of habitat for MacNeill's sootywing requires preserving or establishing suitable *A. lentiformis* host-shrubs and nearby plants of other species that provide adults with nectar. Heliotrope and western purslane may provide nectar, because we have observed sootywings landing and apparently feeding on their flowers (Pratt & Wiesenborn 2009). Here we further examine *H. curassavicum* and *S. verrucosum* as nectar sources for *H. graciellae*. Our objectives were to (1) compare flower visitation between the 2 species when presented alone or together and in direct sunlight or shade and (2) estimate and compare feeding by males and females on *H. curassavicum* nectar.

## MATERIALS AND METHODS

### Study Site

We conducted studies at Cibola National Wildlife Refuge on the Colorado River floodplain in Cibola Valley, La Paz County, Arizona, 37 km south-southwest of Blythe, California. The study site (33°17'N, 114°43'W; elevation 62 m) contains irrigated farm fields converted to wildlife habitat. Historical climate at Blythe includes maximum temperatures averaging 42.4°C during Jul, minimum temperatures averaging 3.1°C during Dec, and rainfall averaging 97 mm yearly and occurring mostly during Dec-Mar and Aug-Sep (DRI 2010). *Atriplex lentiformis* is common at the study site but mostly limited to linear stands downstream of flood-irrigation gates. *Heliotropium curassavicum* is a common groundcover at the study site, whereas *S. verrucosum* is restricted to a few scattered plants.

### Flower Visitation on *H. curassavicum* and *S. verrucosum*

Visitation of *H. curassavicum* inflorescences and *S. verrucosum* flowers by *H. graciellae* adults was examined with potted plants. Plants were grown in 3.8-liter (1-gal) pots from western purslane seed collected at the study site and heliotrope roots collected in the Mojave Desert north of Lancaster, California. We randomized 6 plants of each species into 6 pairs of pots representing all combinations of plant species, species alone (2 plants of same species in pot pair) or together (1 plant of each species in pot pair), and plants in sun or shade projected by a non-flowering mesquite (*Prosopis* sp.) bush. We placed plants <2 m from a clump of *A. lentiformis* with pairs of plants 1 m apart and plants within pairs together with stems intermingled. We counted landings by sootywings on *H. curassavicum* inflorescences or *S. verrucosum* flowers in each plant pair. Four 40-86 min trials were performed during 0832-1133 MST on 14-15 Aug 2007 when air temperatures were 29-38°C. Western purslane flowers (sepals) close every night, so we began trials after flowers had opened. We randomized plants and placements of plant pairs at the start of each trial. We counted inflorescences or flowers on each species in each pair of plants at the start and end of each trial. Soil in pots was kept wet from 13 Aug 2007 until trials were completed.

We examined visitation rates at 2 scales: visitations per plant and per inflorescence or flower (latter termed *index of visitation rate*, Dafni 1992). Visitation rates were calculated by dividing landing frequencies transformed  $\log(f + 1)$  by numbers of plants (1 or 2) in each treatment or average numbers of inflorescences or flowers, transformed by  $\log(n + 1)$  during each trial in each treatment. We compared visitation rates between plant species and between plants in sun or shade with an analysis of variance with trials as blocks (calculated with SYSTAT version 12, Richmond, CA). We also tested interactions between plant species and placing species alone or together and between plant species and placing plants in sun or shade.

### Nectar-feeding on *H. curassavicum*

Nectar-feeding by male and female *H. graciellae* on wild *H. curassavicum* plants was examined by measuring amounts of sugar in inflorescences (Roberts 1979) after landings by sootywings. We timed the duration of each landing on an inflorescence by counting seconds and identified the sootywing's sex by the paler and more-mottled forewings (upper-surface) of females (MacNeill 1970). We marked each visited inflorescence with a partly-unbent paper clip hung on the subtending stem and each supporting plant with a numbered stake. We cut each visited inflores-

cence, counted its open flowers, and collected its nectar by shaking it 30 s in a vial containing 5 mL of water. We performed the study during 1003-1320 MST on 15 Apr 2008, within 2 weeks of the site being flood-irrigated. We froze nectar collections and deposited pressings of *H. curassavicum* and vouchers of adult *H. graciellae* at the Herbarium and the Entomology Museum, University of California, Riverside.

Inflorescence rinses were thawed and centrifuged 10 min at 3K rpm to remove plant debris. We mixed 1 mL of supernatant from each rinse with 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid. After 45 min, we measured sugar concentrations against standards (approximate masses of fructose, glucose, and sucrose) with a spectrophotometer (Cary 100, Varian, Palo Alto, CA) measuring absorbance at 490 nm. Absorbances were linearly related ( $R^2 = 0.95$ ) to concentrations of standards (15, 30, 60, 120 mg/L). We converted sugar concentration to sugar mass,  $\text{mg} = (5 \times 10^{-3})$  (mg/L), based on the 5 mL inflorescence-rinse volume.

We compared landing durations (transformed 1/s) on inflorescences between male and female adults with a *t* test with separate variances for sexes. We similarly compared numbers of flowers and masses of sugar in inflorescences visited by males with those visited by females. We tested dependence of sugar mass on number of flowers and transformed landing duration on each inflorescence with multiple regression within each sex. Dependence of landing duration on sex required separate regressions.

## RESULTS

### Flower Visitation on *H. curassavicum* and *S. verrucosum*

Potted plants produced an average of 13 (range 5-22) inflorescences per plant on *H. curassavicum* and 6 (1-15) flowers per plant on *S. verrucosum*. We observed 49 landings by *H. graciellae* on *H. curassavicum* inflorescences and 42 landings on *S. verrucosum* flowers during 4.5 h.

Sootywing visitation rates on heliotrope inflorescences and western purslane flowers with similar frequencies at both spatial scales (Fig. 1). We did not detect different visitation rates per plant ( $F = 0.60$ ,  $df = 1,23$ ,  $P = 0.45$ ) or per inflorescence or flower ( $F = 0.04$ ,  $df = 1,23$ ,  $P = 0.84$ ) between plant species. Visitation rates per plant on heliotrope compared with western purslane were not influenced by placing species alone or together ( $F = 0.77$ ,  $df = 1,23$ ,  $P = 0.39$ ) or by placing plants in sun or shade ( $F = 0.99$ ,  $df = 1,23$ ,  $P = 0.33$ ). Similarly, visitation rates per inflorescence or flower compared between species were not influenced by placing species alone or together ( $F = 1.5$ ,  $df = 1,23$ ,  $P = 0.23$ ) or by placing plants in sun or shade ( $F = 1.9$ ,  $df = 1,23$ ,  $P = 0.18$ ). Sunlight exposure in-

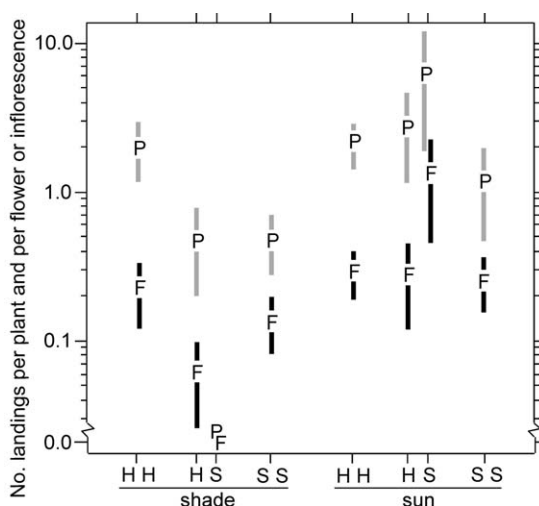


Fig. 1. Frequencies of landings by *Hesperopsis graciellae* adults on *Heliotropium curassavicum* inflorescences and *Sesuvium verrucosum* flowers on potted plants in shade or sun. Letters along X-axis are pairs of plants: H, *H. curassavicum*; S, *S. verrucosum*. Plotted letters are means  $\pm$  SEs ( $n = 4$ ): P, landings per plant; F, landings per flower or inflorescence. Y-axis is log scale.

fluenced overall frequencies of sootywing landings. Across both species, plants in sun were visited more frequently per plant ( $F = 7.6$ ,  $df = 1,23$ ,  $P = 0.011$ ) and per inflorescence or flower ( $F = 8.4$ ,  $df = 1,23$ ,  $P = 0.008$ ) compared with plants in shade (Fig. 1). Greater attraction of plants in sun was particularly evident when species were placed together.

### Nectar-feeding on *H. curassavicum*

We observed 20 males and 13 females of *H. graciellae* visiting inflorescences (all had 2 cymes) on 33 different, wild *H. curassavicum* plants. Landing durations (Fig. 2) of females ( $25 \pm 5.1$  [SE] s) were longer ( $t = 2.51$ ,  $df = 30$ ,  $P = 0.018$ ) than males ( $15 \pm 2.1$  s). Numbers of flowers did not differ ( $t = 0.81$ ,  $df = 31$ ,  $P = 0.42$ ) between inflorescences visited by males ( $8.7 \pm 1.3$  [range 1-21] flowers) or females ( $10 \pm 1.0$  [2-14] flowers). Males and females visited inflorescences similar in size. Masses of sugar in inflorescences (Fig. 2) visited by females ( $0.30 \pm 0.07$  mg) were greater ( $t = 2.30$ ,  $df = 14$ ,  $P = 0.038$ ) than in those visited by males ( $0.13 \pm 0.02$  mg).

Relations between amounts of sugar remaining in inflorescences and landing times differed between sootywing sexes (Fig. 2). In females, sugar masses decreased as landing times increased ( $F = 12.4$ ,  $df = 1,10$ ,  $P = 0.006$ ) but were unrelated to numbers of flowers ( $F = 1.1$ ,  $df = 1,10$ ,  $P = 0.33$ ). In males, sugar masses were not related to either landing times ( $F = 0.5$ ,  $df = 1,17$ ,

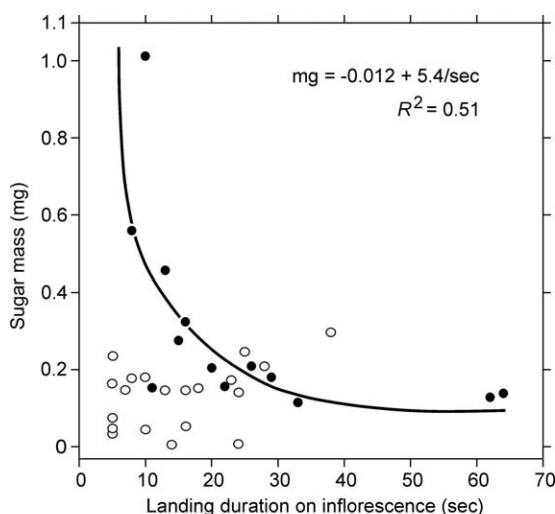


Fig. 2. Masses of sugar in inflorescences on wild *Heliotropium curassavicum* plants after landings by male (open circles) and female (closed circles) *Hesperopsis graciellae* adults.

$P = 0.50$ ) or numbers of flowers ( $F = 3.1$ ,  $df = 1, 17$ ,  $P = 0.10$ ). Sugar masses in inflorescences visited by females decreased asymptotically with increasing landing duration (Fig. 2). The rate of decline in sugar mass decreased as females remained longer on inflorescences.

## DISCUSSION

*Hesperopsis graciellae* adults foraging for nectar did not preferentially land on *H. curassavicum* inflorescences or *S. verrucosum* flowers. Similar visitation rates on these unrelated species, with very-different flower morphologies, agree with our earlier observations of sootywings visiting flowers of various native and naturalized families (Pratt & Wiesenborn 2009). Adult *H. graciellae* appear to be generalist feeders on nectar. Greater visitation rates to plants in sun than in shade suggest sootywings responded visually to flower colors. *Epargyreus clarus* skippers were most attracted to blue, yellow, or magenta flower-models (Swihart 1969). Heliotrope and western purslane plants together displayed all 3 of these floral colors. More landings on flowers in full sun contradict the sootywing's comparatively-low tolerance of direct sunlight (Wiesenborn 1999). Sootywings may have left flowers before exposure-times to full sun (61 s at 29°C, 9 s at 38°C) elicit flight to shade. Stronger visual cues attracting sootywings to flowers in direct sunlight may oppose the species' need for shade.

Amounts of sugar remaining in *H. curassavicum* inflorescences visited by females were greater than in those visited by males. Females

appeared to visit inflorescences with more nectar or with nectar containing higher sugar concentrations. Nectar production likely varied among sampled inflorescences, because they were cut from different plants growing in different soil moistures. Male and female butterflies have been observed foraging differently on plant species producing nectars with different compositions. Female *Lysandra bellargus* Rottemburg lycaenids preferred flowers high in glucose and amino acids, whereas males preferred flowers high in sucrose and total sugars (Rusterholz & Erhardt 2000). Male and female butterflies also have been found to respond differently to floral volatiles. Electroantennographs of *Heliconius melpomene* L. nymphalids measured greater responses by females than males to an assortment of synthetic floral scents (Andersson & Dobson 2003).

Ingestion of nectar from heliotrope inflorescences, suggested by sugar masses that decreased as landing times increased, was detected only in *H. graciellae* females. Larger body size and egg production likely require females to ingest more nectar than males. *Thymelicus lineola* (Ochsenheimer) skippers mature all of their eggs after pupation and produced more eggs when fed sucrose solution (Pivnick & McNeil 1985). Female *T. lineola* also fed longer on sucrose solution and ingested more than males. Amino acids, in addition to carbohydrates, in nectar may affect feeding by adult females compared with males. Acceptance of sugar solutions by female, but not male, nymphalid butterflies was increased when amino acids were added (Erhardt & Rusterholz 1998). The diminishing rate of decline in sugar mass in inflorescences visited by female sootywings suggests nectar ingestion decreased as females became satiated or as inflorescences were emptied. Male sootywings visiting flowers may have been obtaining nectar and searching for mates.

Creating habitat for MacNeill's sootywings should include establishing *H. curassavicum* as a nectar source for adults. Also establishing *S. verrucosum* would provide sootywings with an alternative nectar source when heliotrope is not flowering. Both plant species can flower throughout the sootywing's Apr-Sep flight period (Munz 1974). We have observed one or both species at the study site completely die-back aboveground during dry periods. Western purslane may be more drought-tolerant than heliotrope due to its succulence. A disadvantage of western purslane is its late diurnal flower-opening, preventing sootywings from obtaining nectar during early morning. The ability to irrigate habitat created for *H. graciellae* would enable maintaining plant water-contents high enough for *A. lentiformis* to provide larvae with suitable leaves and for *H. curassavicum* and *S. verrucosum* to provide adults with ample nectar.

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