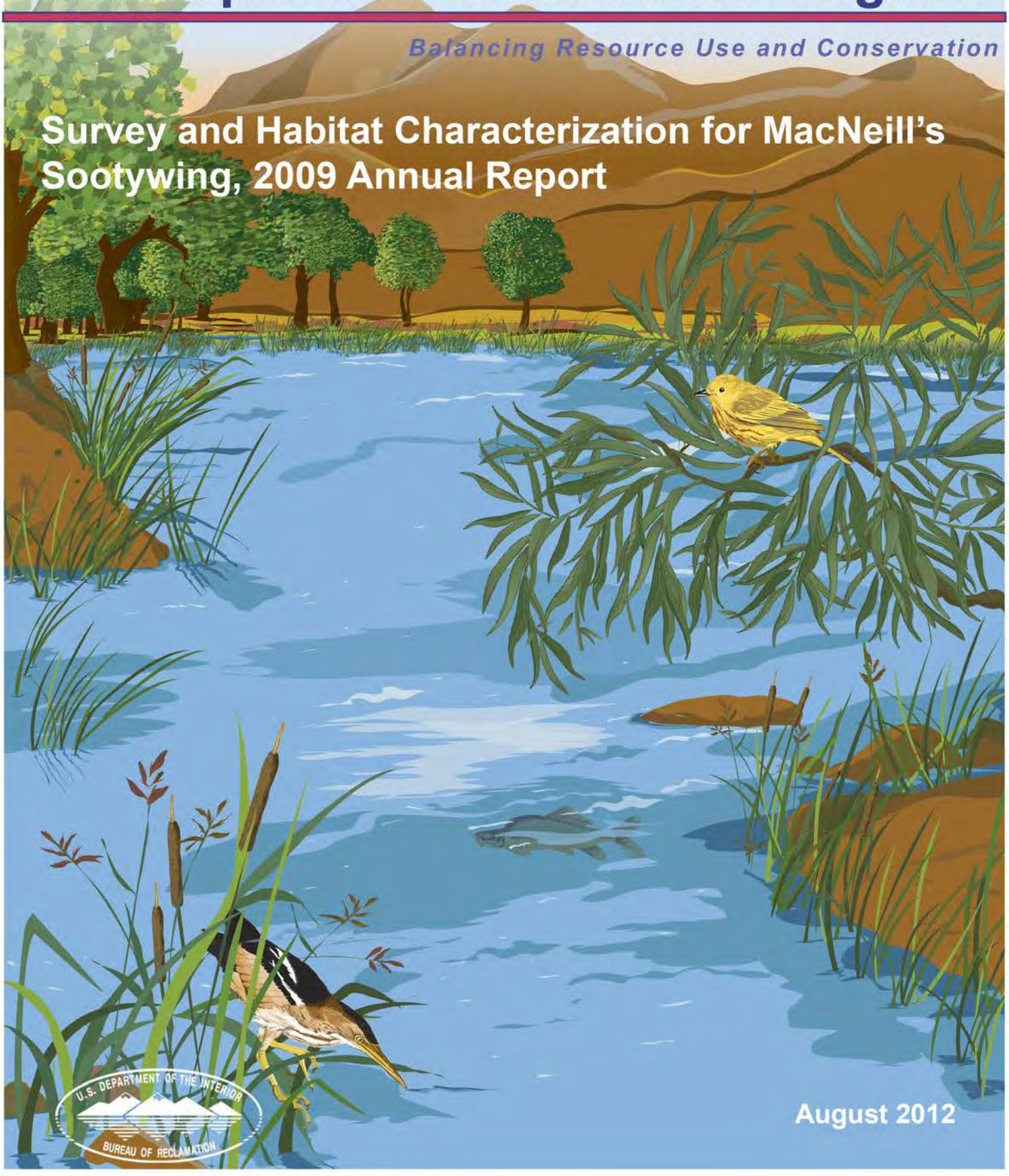
Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation



Survey and Habitat Characterization for MacNeill's Sootywing, 2009 Annual Report

August 2012

Lower Colorado River Multi-Species Conservation Program
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Lower Colorado River Multi-Species Conservation Program

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Lower Colorado River
Multi-Species Conservation Program
Bureau of Reclamation
Lower Colorado Region
Boulder City, Nevada
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Abstract

We performed two studies examining the habitat requirements for MacNeill's sootywing, *Hesperopsis gracielae*. In the first study, we compared oviposition and larval survival on *Atriplex lentiformis*, the sootywing's known host plant, and *Atriplex canescens*, a related species also found along the lower Colorado River. We compared numbers of ovipositions on six potted plants of each species at Cibola National Wildlife Refuge. Sootywings only oviposited (n = 10) on *A. lentiformis*. We compared larval survival on the two plant species by transferring 15 first-instar larvae to three potted plants of each species. Larvae only survived to adults (n = 4) on *A. lentiformis*. Oviposition and survival only on *A. lentiformis* confirms the species as the sootywing's primary host plant. In the second study, we examined the visual and olfactory attraction of sootywing adults to flowers by comparing responses to flower models. Sootywings were most attracted to models presenting blue and yellow together followed by blue models and yellow models. Sootywings were not attracted to floral scent. Adult *H. gracielae* appear to locate flowers primarily by color. Attraction to these two colors agrees with our observations of the plant species producing flowers visited by sootywings.

Introduction

MacNeill's sootywing, *Hesperopsis gracielae* (MacNeill), is a small (wingspread = 23 mm) dark brown butterfly (Lepidoptera: Hesperiidae; Pyrginae) found along the lower Colorado River and near the river along its tributaries in southeastern California, western Arizona, southern Nevada, and southern Utah (MacNeill 1970, Austin and Austin 1980, Scott 1986, Nelson and Anderson 1999). The species is state listed as S1 (critically imperiled) in Nevada and S2 (imperiled) or S3 (rare or uncommon but not imperiled) in Arizona and California. Flights of *H. gracielae* occur from April to October with three generations in southern Nevada (Austin and Austin 1980) and two flights in southeastern California (April and July to October, Emmel and Emmel 1973). MacNeill's sootywing appears to require shade to tolerate the high temperatures where it lives (Wiesenborn 1999).

Larvae of sootywings feed only on quail brush, *Atriplex lentiformis* (Torrey) (Chenopodiaceae), a shrub found in dense clumps along lower Colorado River drainages (Emmel and Emmel 1973). Quail brush fixes atmospheric nitrogen (Malik et al. 1991). Female sootywings oviposit on large (radius > 1.6 m) host plants with high concentrations of water (> 64%) in branches and nitrogen (> 3.2% of dry mass) in leaves (Wiesenborn and Pratt 2008). Sources of nectar for butterflies may limit the sootywing's distribution, because *A. lentiformis* is wind pollinated and does not produce nectar. Other plant species are therefore needed by the skipper for nectar. We have observed sootywings nectar-feeding on eight plant species (Pratt and Wiesenborn 2009):

Heliotrope	*Heliotropium curassavicum*	Boraginaceae	white flowers
Sea purslane	*Sesuvium verrucosum*	Aizoaceae	pink flowers
Arrowweed	*Pluchea sericea*	Asteraceae	purple flowers
Alkali mallow	*Malvella leprosa*	Malvaceae	white-yellow flowers
Screwbean mesquite	*Prosopis pubescens*	Fabaceae	yellow flowers
Honey mesquite	*Prosopis glandulosa*	Fabaceae	yellow flowers
Tamarisk	*Tamarix ramosissima*	Tamaricaceae	white-pink flowers
Common purslane	*Portulaca oleracea*	Portulacaceae	yellow flowers

The objectives of this work task are to: 1) survey the insect and its host plant within the Lower Colorado River Multi-Species Conservation Program (LCR MSCP) boundaries, and 2) determine its habitat requirements. Portions of this work are being performed under a Cooperative Agreement with Gordon Pratt, Department of Entomology, University of California, Riverside.

In the 2008 annual report, we completed the 3-year survey and examined the ingestion of nectar from heliotrope (*Heliotropium curassavicum*) flowers. In this annual report, we confirm *A. lentiformis* as the sootywing's preferred host-plant and examine the skipper's visual and olfactory attraction to flowers. Results from this work task have been used to construct sootywing habitat as part of the LCR MSCP. This work task is integrated with three other LCR

MSCP work tasks: E4: Palo Verde Ecological Reserve (PVER), E5: Cibola Valley Conservation Area (CVCA), and F6: Monitoring MacNeill's Sootywing in Habitat Creation Sites.

During 2009, we performed two additional studies examining the habitat requirements for MacNeill's sootywing. In the first study, we compared oviposition and larval survival on *A. lentiformis*, the sootywing's known host plant, and *Atriplex canescens*. *Atriplex canescens* also is found along the lower Colorado River and is the host plant of *Hesperopsis alphaeus*, a skipper closely related to *H. gracielae*. We therefore were interested in testing whether *H. gracielae* and *H. alphaeus* overlap in their utilization of these two *Atriplex* species.

In the second study during 2009, we examined the visual and olfactory cues that attract *H. gracielae* adults to flowers. We were especially interested in nectar-feeding on *H. curassavicum*, or heliotrope. Heliotrope is a common nectar source for sootywings and produces inflorescences with flowers that change color from yellow to purple.

Methods

Comparison of Oviposition and Survival on *Atriplex* species

We compared oviposition and larval survival on potted *A. lentiformis* and *A. canescens* plants. Six 1-m tall plants of each species were placed within sootywing habitat at Cibola National Wildlife Refuge. Plants were placed in pairs as follows:

. in sun .			. in shade .		
LL	LC	CC	LL	LC	CC

C = *Atriplex canescens*; L = *Atriplex lentiformis*.

Potted plants were placed in the field on 15 June 2009 and retrieved on 29 June 2009. Plants were kept watered. We compared numbers of ovipositions between plant species by counting eggs (Fig. 1) and young larvae.

Figure 1. MacNeill's sootywing eggs on an *Atriplex lentiformis* leaf.

We again used potted plants to compare larval survival between *A. lentiformis* and *A. canescens*. Experiments were performed at Riverside, California. Five first-instar larvae (Fig. 2) were placed on each of three plants of each species, and survival to adult was observed. Trials began on 29 June 2009 and were completed on 31 August 2009.

Figure 2. Early instar MacNeill's sootwing larva (at right with black head) and its feeding damage on an *Atriplex lentiformis* leaf.

Attraction to Floral Color and Scent

Attraction of sootywings to floral color and scent was examined with flower models. Each model was constructed by inserting the threads of a 4-dram glass vial into a hole cut in the center of a 73-mm wide, hexagonal sheet of clear acetate. We placed the acetate on top of a sheet of stiff paper colored over its entire upper surface. We tested blue, yellow, blue and yellow combined, and clear controls. The colors resembled true blue (color 168A) and spectrum yellow (color 55) in Smithe (1974-1981). Models with blue and yellow combined presented three alternating triangles of each color. We added floral scent to half of the models by placing a heliotrope inflorescence with two cymes inside the vial. The inflorescence was placed at the bottom of the vial with its cut stem immersed in water pipetted into all vials. Each flower-model vial was covered with a black plastic cap with a 5-mm diameter hole drilled in its center to allow floral volatiles to escape.

Attraction to flower models was tested at CVCA Phase 4. Flower models were placed in three rows 0.5 m apart in the center of a road that bisected the plot (Fig. 3-4). Eight models representing eight treatments (four color treatments including clear X inflorescence added or not added) were placed 0.2 m apart in each row. We recorded frequencies of sootywings approaching (hovering < 2 cm above model) or landing (on model with wings stopped). We also recorded sexes of sootywings that landed by comparing them with specimens that were pinned and sexed. Males were identified by the upper surface of their wings appearing nearly black, whereas the wings of females are more mottled with brown on their upper surface (MacNeill 1970).

Fig. 3. Flower models on dirt road bisecting CVCA Phase 4 (9 Sep 2009). MacNeill's sootywings approached or landed on models while flying between the *Atriplex lentiformis* shrubs (at the right) and flowering heliotrope plants (at the left).

Fig. 4. Flower models presenting clear, blue, yellow, or both colors together at CVCA Phase 4 (10 Sep 2009). Heliotrope inflorescences were placed within half of the models to test the effects of floral scent.

We conducted four trials on separate days during 0826-1145 PDT on 9-12 September 2009. We randomized placements of treatments within rows and inserted fresh inflorescences at the start of each trial.

Results

Comparison of Oviposition and Survival on *Atriplex* Species

MacNeill's sootyings only oviposited on *A. lentiformis*:

	No. eggs	No. young larvae	sum
Atriplex canescens	0	0	0
Atriplex lentiformis	5	5	10
sum	5	5	10

MacNeill's sootywings therefore preferred to oviposit on *A. lentiformis* compared with *A. canescens* (binomial test, $P < 0.001$).

Larval survival on the two plant species was similar, with larvae (Fig. 4) reaching the adult stage only on *A. lentiformis*:

	No. dying	No. reaching adults	sum
Atriplex canescens	15	0	15
Atriplex lentiformis	11	4	15
sum	26	4	30

Larval survival differed (chi-square = 4.6, 1 df, $P = 0.032$) between *Atriplex* species. MacNeill's sootywings oviposit and develop only on *A. lentiformis*.

Fig. 4. Late instar MacNeill's sootywing larva within a quail brush leaf fold.

Sootywings more frequently approached flower models than landed on them (Fig. 5). Flower models displaying blue and yellow together elicited the most approaches and landings by sootywings. Between models presenting blue or yellow alone, blue models elicited more approaches but equivalent numbers of landings. Adding heliotrope flowers to models, producing floral scent, did not increase numbers of responses by sootywings.

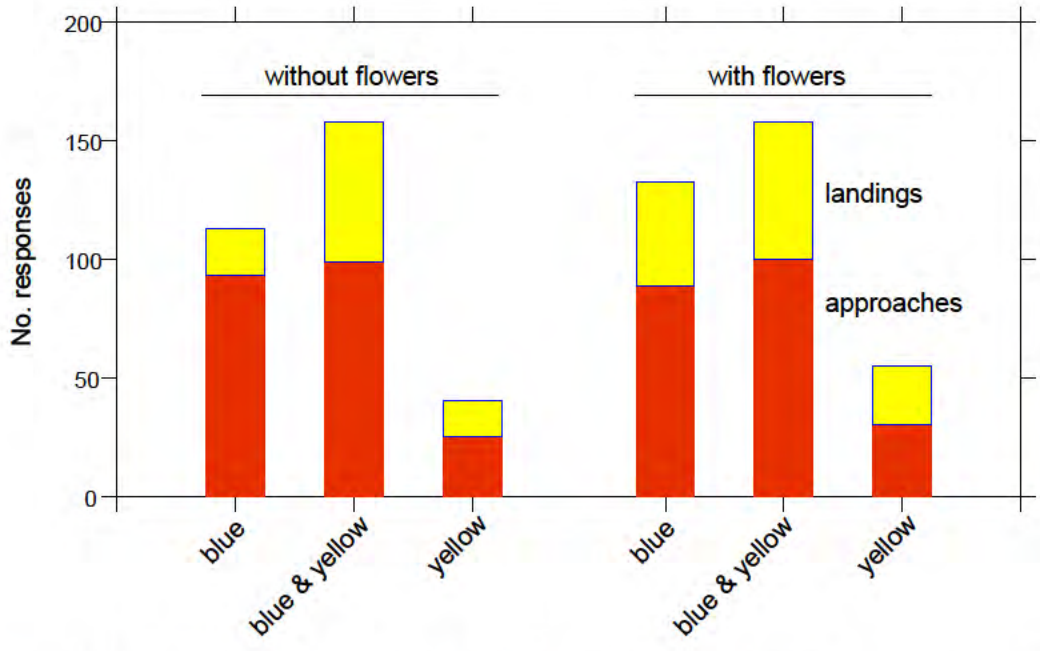

Fig. 5. Numbers of approaches (in red) and landings (in yellow) by MacNeill's sootywings on flower models presenting different colors. Bars on the left represent models without heliotrope scent, and those on the right include heliotrope scent.

Landings on flower models were more frequent by males than females (Fig. 6). Numbers of landings by males, compared with females, did not differ among model colors or between models with or without heliotrope flowers.

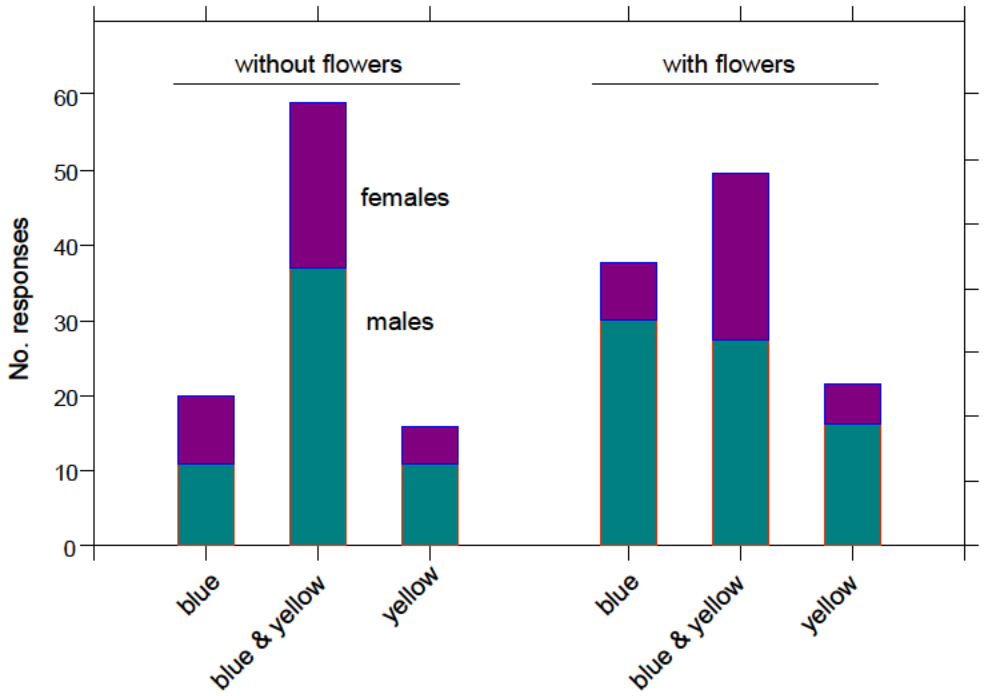

Fig. 6. Numbers of landings by male (in green) and female (in purple) MacNeill's sootywings on flower models presenting different colors. Bars on the left represent models without heliotrope scent, and those on the right include heliotrope scent.

Discussion

Comparison of Oviposition and Survival on *Atriplex* Species

MacNeills' sootywing's appear to specialize on *A. lentiformis*. Evidence for this includes:

1. *A. lentiformis* is indicated as the skipper's host plant in the original species description (MacNeill 1970).
2. *H. gracielae* oviposits on wild *A. lentiformis* shrubs (Wiesenborn and Pratt 2008).
3. *H. gracielae* will not oviposit on the closely related and sympatric *A. canescens*.
4. Larval *H. gracielae* will feed and develop on *A. lentiformis* but not on *A. canescens*.

This work confirms that habitat constructed for the sootywing must contain quail brush.

Specificity for *A. lentiformis* as a host plant also supports the separation of *H. gracielae* from the closely related *H. alphaeus*. MacNeill (1970) split *H. gracielae* from *H. alphaeus* based on morphology, distribution, and host plant. *Hesperopsis alphaeus* occurs throughout the Southwest, but at higher elevations (4,300-7,700 feet) than *H. gracielae*, and utilizes *A. canescens* as its host plant (Stanford 1981). However, this separation is not unanimously accepted, as Scott (1986) shows *H. gracielae* as a subspecies of *H. alphaeus*. As shown here, *H. gracielae* will not oviposit or develop on *A. canescens*.

Attraction to Floral Color and Scent

Responses by MacNeill's sootywings to flower models can be extrapolated to describe foraging on *H. curassavicum* (Fig. 7). Sootywings are attracted to heliotrope flowers primarily by floral colors. Greatest frequencies of responses to models with blue and yellow together suggest sootywings respond most to purple-centered flowers, reflecting blue, and yellow-centered flowers. The white corollas on heliotrope are likely not attractive. Inflorescences with yellow- and purple-centered flowers are more attractive to sootywings than if they were the same size but displayed only one of the two colors. Purple reflected by older flowers elicits more approaches to inflorescences, whereas landings are stimulated by either flower color.

Fig.7. Male MacNeill's sootywing feeding on nectar on heliotrope. Heliotrope inflorescences typically include two cymes, or branches, that support white flowers with colored centers. Flower centers turn from yellow to violet as flowers mature on the cyme from top to bottom.

Flowers on all other plant species reportedly visited by adult *H. gracielae* (Austin and Austin 1980, Pratt and Wiesenborn 2009) reflect blue or yellow. Blue is reflected by violet flowers on *Medicago sativa* L. (Fabaceae) and purplish flowers on *Sesuvium verrucosum* Rafinesque (Aizoaceae), *Pluchea sericea* (Nuttall) (Asteraceae), and *Tamarix ramosissima* Ledebour (Tamaricaceae), and yellow is reflected by yellowish flowers on *Prosopis glandulosa* Torrey (Fabaceae) and *Malvella leprosa* (Ortega) Krapovickas (Malvaceae). Responses to flower

models predict sootywings are most attracted to dichromatic inflorescences on heliotrope followed by blue, and then yellow, reflected from flowers on other species. The assortment of floral scents expectedly produced by these diverse species further indicates sootywings respond to flowers mostly by color. Plant species with flowers or inflorescences reflecting yellow and blue may be preferred nectar sources for MacNeills' sootywings. Habitat constructed for sootywings should provide native plants with flowers reflecting these colors.

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