Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Survey and Habitat Characterization for MacNeill's Sootywing

2010 Annual Report

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

March 2011

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Lower Colorado River Multi-Species Conservation Program

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Multi-Species Conservation Program
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ACRONYMS AND ABBREVIATIONS

CVCA	Cibola Valley Conservation Area
LCR MSCP	Lower Colorado River Multi-Species Conservation Program
m	meter(s)
mL	milliliter(s)
mm	millimeter(s)
PVER	Palo Verde Ecological Reserve
SD	standard deviation
UV	ultraviolet

Symbols

°C	degrees Celsius
µg	microgram(s)
%	percent

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ABSTRACT

Heliotrope (*Heliotropium curassavicum*) is a small desert plant that produces inflorescences visited by MacNeill's sootywing (*Hesperopsis gracielae*), a rare skipper covered by the Lower Colorado River Multi-Species Conservation Program restoration sites. Flowers on heliotrope are white, with yellow centers that turn purple as flowers age, and absorb ultraviolet (UV) light. We measured the amounts of nectar in yellow- and purple-centered flowers and also compared attractions of sootywings to inflorescence models displaying the plant's floral colors. Skippers were more attracted to purple models than yellow models, and white models were less attractive than yellow or purple models. Attraction of sootywings to purple and yellow models corresponded with numbers of purple- and yellow-centered flowers on inflorescences but not with amounts of nectar in individual flowers. Models displaying yellow and purple together, or both colors plus white, elicited fewer approaches but similar numbers of landings than single-color models. Blocking UV light reflected from models greatly increased attractiveness. Adding heliotrope inflorescences to models did not increase attraction. Attraction of MacNeill's sootywings to colors displayed by heliotrope inflorescences supports the importance of the plant as a nectar source for sootywings.

INTRODUCTION

MacNeill's sootywing (*Hesperopsis gracielae*) is a small (wingspread = 23 millimeters [mm]) dark brown butterfly (Lepidoptera:Hesperiidae; Pyrginae) (figure 1) 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*), 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 meters [m]) host plants with high concentrations of water (> 64 percent [%]) 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, therefore, are 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

Figure 1.—Female MacNeill's sootywing on a heliotrope inflorescence at Havasu National Wildlife Refuge.

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 were performed under a Cooperative Agreement with Gordon Pratt, Department of Entomology, University of California, Riverside. Results of 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)
F6: Monitoring MacNeill's Sootywing in Habitat Creation Sites

Funding for Work Task C7 decreased during 2010 to one-half of its original funding, and the Work Task concluded at the end of 2010. Any further work on the sootywing will be performed at the restoration sites under Work Task F6.

In the 2008 Annual Report, we completed the 3-year survey and examined the ingestion of nectar from heliotrope (*Heliotropium curassavicum*) flowers. We confirmed *A. lentiformis* as the sootywing's preferred host plant and examined the skipper's visual and olfactory attraction to flowers in the 2009 Annual Report. In this annual report, we examined in more detail the sootywing's visual and olfactory attraction to heliotrope inflorescences.

Heliotrope inflorescences contain white flowers with centers that change from yellow to purple as flowers age along the cyme (figure 2). Flowers also absorb ultraviolet (UV) light, wavelengths that can affect pollinator behavior. We compared amounts of nectar in young (yellow) flowers and in old (purple) flowers and examined responses of sootywings to the white, yellow, purple, and UV light.

Figure 2.—Male MacNeill's sootywing feeding on a *Heliotropium curassavicum* inflorescence at Cibola National Wildlife Refuge.
Inflorescences contain two cymes. The plant's white flowers have yellow centers that turn purple as flowers age, from the top to the bottom of the cyme. Note that the male has slightly darker wings than the female (figure 1).

METHODS

Attraction to Heliotrope Inflorescence Models

Attraction of sootywings to inflorescence color and scent was examined with inflorescence models. Each model was constructed by inserting a cut pipettor tip into a 15-mm-diameter hole cut in the center of a 6-centimeter-diameter circle of clear acetate. We placed the acetate on top of a sheet of stiff paper colored over its entire upper surface. The pipettor tip was placed on top of a 50-milliliter (mL) plastic centrifuge tube (figure 3).

Figure 3.—Two inflorescence models displaying white, yellow, and purple (left) and yellow and purple (right).
The center of the disk allows volatiles to escape from inflorescences placed into the bottom of the tubes. Models are covered with clear acetate. Reflectance of UV light was blocked with clear plastic that replaced the acetate.

We tested models displaying five patterns of colors: solid white, solid yellow, solid purple, yellow and purple, and yellow, purple, and white. On multiple-color models, we also tested the effects of UV light reflectance and emission of flower volatiles. Reflectance of ultraviolet light was tested by replacing the clear acetate on top of the model with clear plastic that absorbed UV light. The effect of flower volatiles was tested by placing three inflorescences into the bottom of the centrifuge tube with their stems immersed in water. Inflorescences were replaced hourly. We tested models representing the following nine treatments:

1) Solid white
2) Solid yellow
3) Solid purple
4) Yellow and purple squares (see figure 3)
5) Yellow and purple dots on white background (see figure 3)
6) Yellow and purple with inflorescences
7) White, yellow, and purple with inflorescences
8) Yellow and purple with UV light blocked
9) White, yellow, and purple with UV light blocked

Attraction to inflorescence models was tested at CVCA Phase 4. Models were placed in the center of a dirt road that bisects the plot. The north side of the road supports mature *A. lentiformis* shrubs, and the south side of the road supports a large plot of flowering *H. curassavicum* (figures 4–5). Models were placed in three rows 0.5 m apart in the center of the road. Nine models representing the nine treatments were placed 0.2 m apart in each row.

Figure 4.—Patch of heliotrope (lower right corner towards upper left corner) along south side of dirt road bisecting CVCA Phase 4-west. Shrub at center-left is quail brush, the host plant for MacNeill's sootywing larvae.

Figure 5.—MacNeill's sootywings visiting flowers on the patch of heliotrope (figure 4) at CVCA Phase 4-west.
Sootywings flying to and from these flowers landed on the inflorescence models.

We recorded frequencies of sootywings approaching (hovering < 2 centimeters above model) or landing (on model with wings stopped). Sootywings responded to models while crossing the road between the *A. lentiformis* and the *H. curassavicum* (see figure 4). We conducted five trials on separate days during 0746–1135 Mountain Standard Time on July 12–16, 2010. We randomized placements of treatments within rows at the start of each trial. Trials lasted 180–197 minutes, air temperatures were 30–39 degrees Celsius (ºC), and skies were clear or covered 1–50% with hazy clouds.

Nectar Amounts in Yellow- and Purple-Centered Heliotrope Flowers

We estimated amounts of nectar in heliotrope flowers by measuring masses of sugar in water rinses of flowers (Roberts 1979). Adjacent yellow-centered flowers and adjacent purple-centered flowers were cut from 1 cyme (see figure 1) on each of 11 inflorescences. We counted flowers and shook them for 30 seconds in 5 mL of water. Flowers were rinsed during 0700–0730 ST on July 16, 2010, while the field was being irrigated.

Flower rinses were stored frozen, thawed, and centrifuged 5 minutes at 3,000 revolutions per minute to remove plant debris. We mixed 2 mL of supernatant from each rinse with 2 mL of 5% phenol and 5 mL of concentrated sulfuric acid. Sugar concentrations after 1 hour were determined against standards (approximate masses of fructose, glucose, sucrose) with a spectrophotometer (Bausch & Lomb model 20) measuring absorbance at 490 nanometers.

We converted sugar concentration to sugar mass and calculated sugar mass per flower. Sugar masses and sugar masses per flower in yellow-centered flowers were compared with those in purple-centered flowers with a *t*-test paired by cyme.

RESULTS

Attraction to Heliotrope Inflorescence Models

We counted 340 approaches to models and 164 landings on models by MacNeill's sootywings during 15.3 hours. Sootywings sometimes approached or landed on more than one model as they flew among them.

Model color influenced numbers of approaches and landings (figure 6). Sootywings responded more frequently to purple models than yellow models, and either color was more attractive than white, as the sootywings approached white

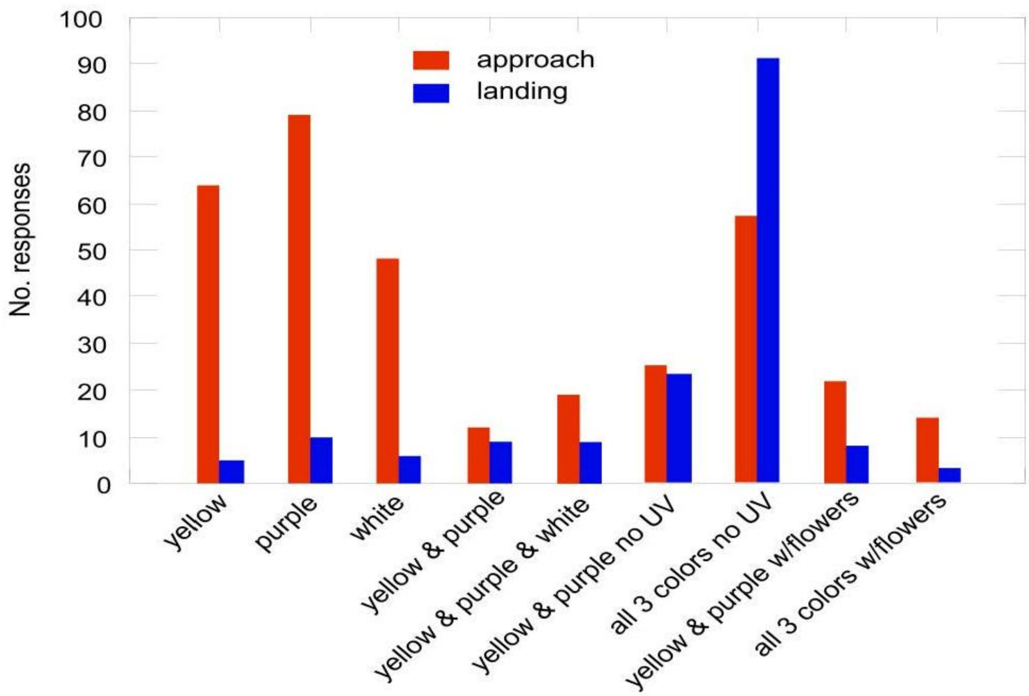

Figure 6.—Numbers of approaches to models of heliotrope inflorescences, and landings on models, by MacNeill's sootywings at CVCA Phase 4-west.

models less frequently than yellow or purple. Responses by sootywings to models differed greatly when colors were presented alone compared to when colors were presented together. Single-color models were approached more often than multiple-color models. The numbers of landings on single-color and multiple-color models were similar. Colors of multiple-color models also influenced sootywing behavior. Multiple-color models reflecting white were more frequently approached, but not landed upon.

Ultraviolet reflectance strongly affected responses of sootywings to multiple-color models (see figure 6). Models absorbing UV light elicited four times more responses than models reflecting UV light. Blocking UV light also increased numbers of landings more than numbers of approaches. Multiple-color models reflecting white but absorbing UV light were especially attractive. Models reflecting yellow, purple, and white, but not UV light, elicited 56% of landings by sootywings on all models. Sootywings did not respond to *H. curassavicum* inflorescences added to multiple-color models (see figure 6). Placing inflorescences within multiple-color models did not affect frequencies of approaches or landings.

Nectar Amounts in Yellow- and Purple-Centered Heliotrope Flowers

Heliotrope cymes (*n* = 11) supported more yellow-centered flowers (1–2 flowers per cyme) than older, purple-centered flowers (3–6 flowers per cyme). Young, yellow-centered flowers also contained high masses of sugar per flower (27 ± 9 micrograms [µg]) than purple-centered flowers (8.9 ± 3.8 µg). When masses of sugar were combined across same-colored flowers on each cyme, masses in yellow-centered flowers (35 ± 17 [SD] µg) were similar to those in purple-centered flowers (42 ± 20 µg). Amounts of nectar in heliotrope flowers decline as flowers age, and turn color, along the cyme.

DISCUSSION

Attraction of MacNeill's sootywings to purple (bluish-violet), followed by yellow, single-color models, agrees with the relative attractiveness of blue and yellow to the silver-spotted skipper (*Epargyreus clarus)*, the only other skipper whose attraction to color has been studied (Swihart 1969). More approaches to, or landings on, purple than yellow also corresponded with abundances of yellow- and purple-centered flowers on heliotrope inflorescences. It contrasted, however, with the greater amount of nectar found in yellow-centered flowers. Yellow-centered flowers occur at the top of heliotrope inflorescences and point upwards. Sootywings usually land on top of cymes and initially feed on yellow-centered flowers. They will then walk around the top of the inflorescence and feed on other yellow-centered flowers and younger, purple-centered flowers.

Models displaying more than one color (see figure 3) elicited fewer approaches than single-color models. Patterns of multiple-color models may have decreased responses because model pattern can influence attraction to insects, including Lepidoptera. Numbers of approaches by sootywings increased when yellow and purple were surrounded by white, similar to heliotrope inflorescences.

Reflection of UV light greatly affected the attraction of sooywings to models. Visual contrast of UV-absorptive models against the UV-reflective soil at the site likely resembled the contrast of UV-absorptive heliotrope flowers against UV-reflective foliage. Frequencies of landings increased more than approaches when UV light was blocked. This suggests that sootywings are initially attracted to inflorescences in response to visible light and subsequently land in response to lack of UV light. Landings were most frequent on models that most resembled heliotrope inflorescences, purple and yellow dots surrounded by white that absorbed UV light. Blocking the ultraviolet reflected from white may have increased the contrast of yellow and purple dots against their paper background.

Lack of response by sootywings to floral volatiles from heliotrope agrees with previous experiments that showed greater responses by butterflies to floral color. The relatively crude models examined may have contributed to the lack of olfactory response by sootywings: (1) floral volatiles from nearby heliotrope plants may have masked volatiles from models containing inflorescences and (2) models displayed more color, but approximately equivalent amounts of floral volatiles, than heliotrope inflorescences. Sootywings may respond more to volatiles, especially from nectar, if they are more closely combined with floral color.

Attraction of MacNeill's sootywings to models mimicking heliotrope inflorescences suggests the plant is an important source of nectar for the skipper. It displays the two colors, yellow and purple (or blue), most attractive to skippers, and provides nectar in a tubular flower that corresponds with the sootywing's straw-like feeding apparatus. Although sootywings have been observed on flowers on other plant species, as listed above, they are most often seen on heliotrope. Aggregation of sootywings on patches of heliotrope provides a simple method of surveying the species. Heliotrope should be established, from volunteering or planting, at all of the LCR MSCP restoration plots intended to establish MacNeill's sootywings.

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