Survey and Habitat Characterization for MacNeill’s Sootywing, 2007 Annual Report
Lower Colorado River Multi-Species Conservation Program
Steering Committee Members

**Federal Participant Group**
Bureau of Reclamation  
U.S. Fish and Wildlife Service  
National Park Service  
Bureau of Land Management  
Bureau of Indian Affairs  
Western Area Power Administration

**Arizona Participant Group**
Arizona Department of Water Resources  
Arizona Electric Power Cooperative, Inc.  
Arizona Game and Fish Department  
Arizona Power Authority  
Central Arizona Water Conservation District  
Cibola Valley Irrigation and Drainage District  
City of Bullhead City  
City of Lake Havasu City  
City of Mesa  
City of Somerton  
City of Yuma  
Electrical District No. 3, Pinal County, Arizona  
Golden Shores Water Conservation District  
Mohave County Water Authority  
Mohave Valley Irrigation and Drainage District  
Mohave Water Conservation District  
North Gila Valley Irrigation and Drainage District  
Town of Fredonia  
Town of Thatcher  
Town of Wickenburg  
Salt River Project Agricultural Improvement and Power District  
Unit “B” Irrigation and Drainage District  
Wellton-Mohawk Irrigation and Drainage District  
Yuma County Water Users’ Association  
Yuma Irrigation District  
Yuma Mesa Irrigation and Drainage District

**California Participant Group**
California Department of Fish and Game  
City of Needles  
Coachella Valley Water District  
Colorado River Board of California  
Bard Water District  
Imperial Irrigation District  
Los Angeles Department of Water and Power  
Palo Verde Irrigation District  
San Diego County Water Authority  
Southern California Edison Company  
Southern California Public Power Authority  
The Metropolitan Water District of Southern California

**Nevada Participant Group**
Colorado River Commission of Nevada  
Nevada Department of Wildlife  
Southern Nevada Water Authority  
Colorado River Commission Power Users  
Basic Water Company

**Native American Participant Group**
Hualapai Tribe  
Colorado River Indian Tribes  
The Cocopah Indian Tribe

**Conservation Participant Group**
Ducks Unlimited  
Lower Colorado River RC&D Area, Inc.

**Other Interested Parties Participant Group**
QuadState County Government Coalition  
Desert Wildlife Unlimited
Abstract.
We continued to locate host plants (*Atriplex lentiformis*) and eggs, larvae, and adults of MacNeill's sootywing (*Hesperopsis gracielae*) by surveying the lower Colorado River between the northern boundary of Imperial National Wildlife Refuge and the Southerly International Boundary with Mexico. Stands of host plants were found at 21 additional sites that were entered onto Reclamation's Regional GIS. Sootywings were found at 11 of these localities. We continued observing plant species used by sootywings for nectar. A seventh plant species used for nectar was identified—the weedy succulent *Portulaca oleracea* (Portulacaceae). We also compared frequencies of nectaring on potted *Heliotropium curassavicum* (Boraginaceae) and *Sesuvium verrucosum* (Aizoaceae), two species observed as nectar sources during 2006. Nectarings per plant did not differ between plant species. Nectarings per flower were greater on *S. verrucosum*, the species with fewer flowers per plant. We continued observing plant species used by sootywings for nectar. A seventh plant species used for nectar was identified—the weedy succulent *Portulaca oleracea* (Portulacaceae). We also compared frequencies of nectaring on potted *Heliotropium curassavicum* (Boraginaceae) and *Sesuvium verrucosum* (Aizoaceae), two species observed as nectar sources during 2006. Nectarings per plant did not differ between plant species. Nectarings per flower were greater on *S. verrucosum*, the species with fewer flowers per plant. We completed a study of host-plant selection by ovipositing sootywings begun in 2006 at Cibola National Wildlife Refuge. The effects of plant size (canopy radius), plant water content, and leaf water content on host acceptance were tested. Percentages of plant water and leaf nitrogen were positively correlated. Acceptance of plants was most-influenced by plant size and leaf nitrogen-content acting simultaneously. All plants (n = 9 of 39 plants sampled) that exceeded 1.6 m in canopy radius, 64% in water content, and 3.2% in leaf nitrogen received eggs. We present preliminary recommendations for restoring sootywing habitat based on our survey and study results.

Introduction.
MacNeill’s sootywing, *Hesperopsis graciellae* (MacNeill), is a small (wingspread 0.23 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 (Scott 1986, Nelson and Anderson 1999). Flights of *H. graciellae* 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). Sootywings are more rare than would be expected based on the occurrence of their host plant (Austin and Austin 1980), and this rarity has caused the species to be listed as S1 (critically imperiled) in Nevada (NHHP 2007) and S2 (imperiled) or S3 (rare or uncommon but not imperiled) in California (CDFG 2009) and Arizona (AZGFD 2009). 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 species for nectar.

This work task has two objectives: 1) to survey the insect and its host within the MSCP boundaries, and 2) to determine its habitat requirements. Surveys will be used to gauge the species’ rarity within the project area and identify populations that can be expanded by habitat creation. Determining the sootywing's habitat requirements where it now lives will enable creating additional habitat. This work is being performed under a Cooperative Agreement between Reclamation and Gordon Pratt, Department of Entomology, University of California, Riverside.
Study Area.
Surveys are being conducted within the historical floodplain of the lower Colorado River from the upstream end of Lake Mead to the Southerly International Boundary (SIB) with Mexico. The river is being surveyed in three sections: Parker Dam to lower end of Cibola National Wildlife Refuge (Cibola NWR) during 2006, upper end of Imperial NWR to the SIB during 2007, and upstream end of Lake Mead to Parker Dam during 2008. Survey permits have been provided by Lake Mead National Recreation Area (managed by National Park Service), Bureau of Land Management, and Cibola and Imperial NWR's (managed by Fish and Wildlife Service). Permission from other landowners or managers will be obtained as needed. Private property will not be surveyed. Most of the research determining habitat requirements is being conducted at Cibola NWR.

Methods.

1) Surveys for sootywings, their host plants, and nectar sources. Surveys are being conducted by recording GPS coordinates of stands of quail brush and by searching plants for sootywing eggs, larvae, and adults. Quail brush stands are being repeatedly sampled for sootywings if possible. Plants with flowers being visited by sootywings for nectar are being collected and identified.

2) Preference of sootywings for plant species providing nectar. We identified six plant species used by sootywings for nectar during 2006. We began an experiment during 2007 to test for preference between two of these species, *Heliotropium curassavicum* (Boraginaceae) and *Sesuvium verrucosum* (Aizoaceae). These plants are common at Cibola NWR, and sootywings have repeatedly been seen nectaring on them. Six potted plants of each species were grown from seed collected at Cibola NWR. Plants were randomized into four treatments: (1) species alone in sun, (2) species alone in shade, (3) species together in sun, (4) species together in shade. Open inflorescences on plants were counted. Plants were observed for nectaring by sootywings during three sequential trials. Nectarings per plant and per flower were recorded and analyzed by an AOV testing sun versus shade, plant species, species alone or together, and 2-way interactions. Trials were blocks.

3) Association between host plant size, water content, and nitrogen content. We completed this study begun in 2006 by sampling seven additional *A. lentiformis* shrubs for sootywing eggs at Cibola NWR. A total of 39 shrubs with or without sootywing adults were alternately sampled on three dates. Plant diameters were measured, and plant samples were taken and analyzed for water and nitrogen contents. Nitrogen content was determined as Total Kjeldahl Nitrogen. We measured partial correlations between plant measurements. Association between plant acceptance (oviposition on plant), plant radius, and plant water and nitrogen contents were tested with logistic regression. Relation between egg density and water and nitrogen contents on accepted plants (those with 1 or more eggs) was tested with linear regression. All regressions included sampling date as an indicator variable.

Results.

1) Surveys for sootywings, their host plants, and nectar sources. Stands of *A. lentiformis* were found at 21 localities between the northern boundary of Imperial NWR and the Southerly International Boundary with Mexico. Sootywing eggs, larvae, pupae, or adults were found at 11 of these localities. Quail brush now has been found at 61 sites south of Parker Dam, and 36 of
these sites support sootywings. Survey results for both 2006 and 2007 are shown below (maps 1 through 5), from north to south. Of interest is the site 'type local', on map 1 below, where the species was first described in 1970. We have not found sootywings at this locality during 2006 or 2007.
We observed sootywings nectaring on common purslane along the river south of Yuma. This brings the list of plants used by sootywings for nectar to seven:

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Scientific Name</th>
<th>Family</th>
<th>Flower Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heliotrope</td>
<td><em>Heliotropium curassavicum</em></td>
<td>Boraginaceae</td>
<td>white flowers</td>
</tr>
<tr>
<td>Sea purslane</td>
<td><em>Sesuvium verrucosum</em></td>
<td>Aizoaceae</td>
<td>pink flowers</td>
</tr>
<tr>
<td>Arrowweed</td>
<td><em>Pluchea sericea</em></td>
<td>Asteraceae</td>
<td>purple flowers</td>
</tr>
<tr>
<td>Alkali mallow</td>
<td><em>Sida rhombifolia</em></td>
<td>Malvaceae</td>
<td>white flowers</td>
</tr>
<tr>
<td>Honey mesquite</td>
<td><em>Prosopis glandulosa</em></td>
<td>Fabaceae</td>
<td>yellow flowers</td>
</tr>
<tr>
<td>Tamarisk</td>
<td><em>Tamarix ramosissima</em></td>
<td>Tamaricaceae</td>
<td>white-pink flowers</td>
</tr>
<tr>
<td>Common purslane</td>
<td><em>Portulaca oleracea</em></td>
<td>Portulacaceae</td>
<td>yellow flowers</td>
</tr>
</tbody>
</table>

2) Preference of sootywings for plant species providing nectar. We observed 46 nectarings by sootywings on potted *H. curassavicum* and 41 nectarings on potted *S. verrucosum*. Numbers of nectarings per plant did not differ between plant species ($F = 0.18; \text{df} = 1,15; \text{P} = 0.68$). More sootywings nectared on plants in sun than in shade ($F = 7.1; \text{df} = 1,15; \text{P} = 0.018$). Plant species together or alone did not affect numbers of nectarings ($F = 0.16; \text{df} = 1,15; \text{P} = 0.69$).

![Bar chart showing mean nectarings per plant in sun and shade for *Heliotropium* and *Sesuvium*](chart.png)

Inflorescences were more abundant per plant on *H. curassavicum* (8.3 inflorescences per plant) than on *S. verrucosum* (4.6 inflorescences per plant). Numbers of nectarings per flowers were greater on *S. verrucosum* than on *H. curassavicum* ($F = 6.8; \text{df} = 1,15; \text{P} = 0.020$). Light had a similar affect as above.
3) Association between host plant size, water content, and nitrogen content. Percentages of plant water and leaf nitrogen were positively correlated (partial $r = 0.44; t = 3.0; \text{df} = 36; P = 0.003$).

Twenty-three of the 39 quail brush shrubs sampled on the three dates supported at least one sootywing egg. Plant acceptance was most affected by concentrations of plant water and leaf nitrogen. Female sootywings were more likely to accept *A. lentiformis* shrubs with larger canopy radius ($t = 2.4; \text{df} = 35; P = 0.017$) and greater concentration of leaf nitrogen ($t = 2.1; \text{df} = 35; P = 0.033$).
On shrubs with at least one egg, numbers of eggs were not influenced by percentages of plant water \( (t = 1.8; \ df = 9; \ P = 0.14 \text{ on } 24 \text{ May } 2006; \ t = 2.0; \ df = 9; \ P = 0.078 \text{ on } 29 \text{ June } 2006 \text{ and } 5 \text{ June } 2007) \) or leaf nitrogen \( (t = 1.0; \ df = 9; \ P = 0.33 \text{ on } 24 \text{ May } 2006; \ t = 1.6; \ df = 9; \ P = 0.15 \text{ on } 29 \text{ June } 2006 \text{ and } 5 \text{ June } 2007) \). Dates were split due to varying plant water contents. All plants \( (n = 9 \text{ of } 39 \text{ plants sampled}) \) that exceeded 1.6 m in canopy radius, 64% in water content, and 3.2% in leaf nitrogen received eggs.

**Discussion.**

We now have surveyed most of the lower Colorado River that is bounded by floodplain and most likely to support quail brush. Eggs, larvae, or adults have been observed at 59% of *A. lentiformis* stands. Roughly one third of quail brush along the river does not support sootywings. An example of this disparity is the absence of sootywings at the Parker Strip location where the species was first described (MacNeill 1970). The presence of host plants alone does not assure the presence of the butterfly. In contrast, we have found the sootywing to be geographically widespread. We have found them from the Bill Williams River south to the southerly Mexican border. The largest population of *H. gracielae* found during 2006 and 2007 is at Cibola NWR. This population has allowed us to study phenology, adult behavior, and oviposition on plants.

We have not seen a pattern in the sootywing's use of plant species providing nectar. It uses flowers of a range of colors (e.g. white, pink, yellow) on plants that are native (e.g. sea purslane) or introduced (e.g. tamarisk), in a variety of plant families, and growing as shrubs (e.g. mesquite) or ground cover (e.g. heliotropium). The use of tamarisk is especially perplexing given the plant's abundance compared with the rarity of sootywings.

Differences between *A. lentiformis* plants were associated with egg deposition by sootywings. We found plant size and plant nitrogen content most associated with oviposition. These two factors appear to influence plant acceptance in a dichotomous fashion. Plants large enough, and with high enough levels of nitrogen, are acceptable to females. Sootywings likely respond visually to plant size, and chemically (via taste receptors in their feet) to plant nitrogen. Insects generally have greater survival and fecundity on plants with higher nitrogen. Female sootywings would be expected to invest eggs on plants with greater likelihood of offspring survival. Plant...
size, water content, and nitrogen content appear to be important variables to consider in creating habitat.

**Recommendations.**
The river north of Parker Dam remains to be surveyed during 2008 for quail brush and sootywings. We also need to better understand the supply of nectar provided to sootywings by the various plant species. Observing butterflies on flowers does not equate with butterflies obtaining nectar from flowers. We plan to measure variation in nectar among plants within species and among species during 2008. We also will continue our comparison of nectaring on potted plants.

Although we have not completed our three-year survey and studies on MacNeill's sootywing, we can provide preliminary instructions for creating sootywing habitat:

1) Plant native *Atriplex lentiformis* as host plants. There is enough quail brush growing along the river to provide seeds or transplants for planting. Previous revegetation plants should be avoided. Plants will not become suitable as sootywing hosts until they reach a height of 1.6 m. We have not detected a minimum patch size required by sootywings. However, other studies have estimated minimum patch sizes of 2-6 ha for preserving butterflies (Crone and Schultz 2003).

2) Maintain host-plant water content above 64% during April 1 through November 30 when sootywings are flying or larvae are feeding. We suggest a deep irrigation during March followed by monthly monitoring of plant percent water. Additional irrigation should be performed if plant water drops below this level. Maintaining adequate water content should produce adequate leaf-nitrogen content, because these two quantities are positively correlated in quail brush. Leaf nitrogen content can be measured if sootywings fail to become established. If leaf-nitrogen concentration is low (< 3.2%), fertilizer containing phosphate, potassium, and nitrogen can be added to increase nitrogen uptake and fixation.

3) Establish native plants amongst the quail brush to provide nectar during April 1 through September 30 when sootywings are flying. Honey or screwbean mesquite may provide nectar during spring, but other plants will be needed to supply nectar through September 30. We suggest a mixture of heliotrope, *Heliotropium curassavicum* (Boraginaceae), and sea purslane, *Sesuvium verrucosum* (Aizoaceae). These perennial ground-covers flower (Munz 1974) from March to October (*H. curassavicum*) or April to November (*S. verrucosum*). Planting patches of both plants at each site will help ensure that flowers are present during years with different rainfalls. Nectar plants should be watered by the irrigations of quail brush.

Insects are strong dispersers. Dispersal by sootywings should enable them to colonize new habitat on their own. If suitable habitat fails to become colonized, then sootywings from nearby populations can be transplanted.
Literature Cited.


Wiesenborn, W. D. 1999. Sunlight avoidance compared between Hesperopsis gracielae (MacNeill) (Lepidoptera: Hesperiidae) and Brephidium exilis (Boisduval) (Lepidoptera: Lycaenidae). Pan-Pacific Entomologist 75(3):147-152.