Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Monitoring of the MacNeill's Sootywing Skipper and its Habitats

2014 Annual Report

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Monitoring of the MacNeill's Sootywing Skipper and its Habitat

2014 Annual Report

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ACRONYMS AND ABBREVIATIONS

bp	base pair
Cibola NWR	Cibola National Wildlife Refuge
COI	cytochrome oxidase I
CVCA	Cibola Valley Conservation Area
DNA	deoxyribonucleic acid
eDNA	environmental deoxyribonucleic acid
HMM	Hart Mine Marsh
LCR	lower Colorado River
LCR MSCP	Lower Colorado River Multi-Species Conservation Program
mph	mile(s) per hour
n	sample size
PCR	polymerase chain reaction
PVER	Palo Verde Ecological Reserve
Reclamation	Bureau of Reclamation
RH	relative humidity
sootywing	MacNeill's sootywing skipper (*Pholisora gracielae* = *Hesperopsis gracielae* [MacNeill])

Symbols

°F	degrees Fahrenheit
≥	greater than or equal to
≤	less than or equal to
$\bar{X}$	mean
%	percent
±	plus or minus
α	statistical significance

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Attachment

1 MacNeill's Sootywing Skipper (*Pholisora gracielae* = *Hesperopsis gracielae* [MacNeill]) Monitoring Data Sheet

2 Maps of Study Areas

EXECUTIVE SUMMARY

A monitoring protocol for the MacNeill's sootywing skipper (*Pholisora gracielae* = *Hesperopsis gracielae* [MacNeill]) (hereafter sootywing), a small, dark-colored butterfly, was designed and tested along the lower Colorado River as part of the Lower Colorado River Multi-Species Conservation Program (LCR MSCP). The protocol included searching for eggs, larvae, and adults to determine presence/absence at various plots. Eight plots were surveyed for sootywings in May, June, July, and August 2014 along the Colorado River from just south of Laughlin, Nevada, downriver to the Cibola National Wildlife Refuge (Cibola NWR) south of Blythe, California. Four of these plots (Palo Verde Ecological Reserve (PVER)-4, PVER-6-north, Cibola Valley Conservation Area, and Hart Mine Marsh [HMM]) were in LCR MSCP conservation areas. Four other sites, located off LCR MSCP conservation areas, were at Davis Dam near Laughlin, Nevada; Needles and Blythe, California; and the Cibola NWR, Arizona, were also surveyed to validate the method and to verify presence of sootywings in other areas. Adults were detected at seven plots (when a revisit to the Needles (NW) plot was included), eggs at four plots, and larvae at five plots. Sootywings were detected at PVER-4 in all four months. Sootywings at the Cibola Valley Conservation Area, the Cibola NWR, and HMM were detected in three months, while detections at PVER-6-north and Blythe NE (NE) occurred in two months. Sootywings at Davis Dam SE (NW) and Needles (NW) were only detected in a single month. All three life stages were observed at three plots (PVER-4, PVER-6-north, and the Cibola NWR). At Davis Dam SE (NW), only a single egg was detected; at Needles (NW), adults were detected once, but this was during a revisit. In May, four out of eight plots were positive for sootywings; in June, two out of eight plots; in July, five out of eight plots; and in August, eight out of eight plots were positive for sootywings.

Habitat information was also collected at plots and included measures of quailbush (*Atriplex lentiformis*) (sootywing larval host plant) height, width, percent dry (indicator of lushness), and lux (indicator of amount of shade); nectar plant floral abundance and diversity of flower sources; soil moisture; air temperature; and relative humidity. Quailbush lushness was the only characteristic that differed significantly between plants occupied by early life history stages and unoccupied plants. Quailbush was recorded as a possible new nectar plant for sootywings.

Repeated surveys at a known sootywing plot were used to estimate the length of survey time needed to detect sootywings on occupied plots. Data indicated that 1 hour of survey time (at the appropriate time of day) would detect adult sootywings in 90% of sampled intervals. Examination of the time of day and effort needed for detection suggested a lull in adult sootywing activity that occurred in late afternoon (approximately 14:00–16:00). This break in activity seemed to correspond with peaks in air temperature.

During some months, limited detection of sootywings occurred. An alternative sampling method (supported by the Bureau of Reclamation's Science and Technology Program) using deoxyribonucleic acid (DNA) was trialed to design sootywing primers for detection of environmental DNA (eDNA). Sootywing samples were analyzed using universal DNA primers to obtain an initial mitochondrial gene cytochrome oxidase I sequence that could be used to design sootywing-specific polymerase chain reaction (PCR) primers. Primers were tested against known sootywing samples and a moth sample. The moth sample gave no PCR product, but both sootywing samples gave positive PCR results.

Detection bias for sootywings can be reduced by sampling for a suitable length of time, at the appropriate time of day, with repeated sampling at quailbush plots. Sampling for eDNA has the potential to identify occupied sites where visual detections are challenging.

INTRODUCTION

The MacNeill's sootywing skipper (*Pholisora gracielae = Hesperopsis gracielae* [MacNeill]) (hereafter sootywing), is a small (wingspan 0.79 to 1.25 inches [20 to 32 millimeters]), dark-colored butterfly endemic to the lower Colorado River (LCR) system (Pratt and Wiesenborn 2011). Larvae of sootywings can only complete their development on quailbush (*Atriplex lentiformis*) (Wiesenborn and Pratt 2008). Adult sootywings are mostly dependent on additional plant species for nectar (Wiesenborn and Pratt 2010).

The sootywing is the only invertebrate covered by the Lower Colorado River Multi-Species Conservation Program (LCR MSCP). The LCR MSCP is expected to facilitate a balance between anthropogenic use of river resources and the conservation of native species and their habitats (http://www.lcrmscp.gov/general_program.html).

The purpose of this study was to design and test a monitoring method to detect the sootywing and its habitats. In many cases, monitoring questions that need to be answered are basic and include how and where to count/detect sootywings, what the active period for adults is, and how long a patch of habitat should be searched. Monitoring biological populations is often difficult because of their complexity (several different life stages) and variability in abundance. This variability can be between patches of habitat or over a season at a given habitat patch. Past studies indicated that sootywings exhibit characteristics of rarity and may have erratic population abundance changes that might make monitoring challenging (Wiesenborn 2009, 2010). Sootywing habitat also appeared in past studies to be subject to changes that make plots more or less suitable over relatively short periods of time (Wiesenborn 2012, 2017). Precipitation and/or irrigation patterns have strong effects on habitat suitability, and this may change over the course of a season. It is likely that characterizing habitat and detecting sootywings are both important in the design of a monitoring scheme. Sampling methods may also bias observations in which one category, such as presence, may be more or less probable than another category, such as absence.

The potential rarity of the sootywing makes development of alternative sampling methods desirable. Locating low-abundance species is difficult and requires costly and time-consuming efforts to increase detection probability. Alternative sampling methods are, however, available. Analyzing for deoxyribonucleic acid (DNA) is considered a sensitive forensics approach that can be used to detect a species without direct observation. Organisms shed genetic material into the environment, and this DNA is known as environmental DNA (eDNA). This eDNA can be used to indicate the recent presence of a target species through the recognition of diagnostic DNA fragments (Dejean et al. 2011; Waits and Paetkau 2005). eDNA is currently most useful for presence monitoring and studies. The persistence of eDNA from butterflies in terrestrial environments is unknown.

A monitoring plan was created based on the life history of sootywings in conjunction with pertinent information derived from the literature. The monitoring protocols were then tested by performing surveys over 4 months at a set of plots along the LCR. Information from these surveys was collected, in part, to develop techniques to decrease the detection bias of sootywings.

LIFE HISTORY

Adult sootywings (figure 1) can be observed from March to October (Emmel and Emmel 1973; Nelson and Andersen 1999).

Figure 1.—Adult sootywing at the Palo Verde Ecological Reserve in 2014.

Eggs and caterpillars are found on quailbush plants. Eggs (figure 2) are less than 1 millimeter in diameter and are described as spherical and heavily sculptured with a ridged chorion and reddish brown in color (Wiesenborn and Pratt 2008). However, the eggs are described as ivory-white in color by Emmel and Emmel (1973). Pratt and Wiesenborn (2011) further indicate that sootywing eggs from the Cibola National Wildlife Refuge (Cibola NWR) north to Overton are dark in color, while those from the Imperial National Wildlife Refuge south to Baja California are a cream or ivory color. Eggs (figure 2) are often laid singly close to the mid-vein of a quailbush leaf and can be found on both the upper and undersides of leaves.

Figure 2.—Sootywing egg at the Palo Verde Ecological Reserve (PVER)-6-north in 2014.

Caterpillars are described as blue to yellow green, covered with tiny white dots, with the head black and heart shaped (figure 3); their size ranges by age (up to 2 millimeters). The head is covered with short, white hair (Allen et al. 2005) and is large relative to the thorax, creating a constriction between the head and the first segment. Caterpillar nests (unlike those of some moth caterpillars) are devoid of droppings (Allen et al. 2005). Sootywing caterpillars are foliage feeders, and damage often occurs only on the surface portion of quailbush leaves. After hatching, caterpillars can be found crawling and feeding on the quailbush. The caterpillar also folds a portion of a leaf over itself when young and, when older, will often make a protective tent of two to three leaves, which it inhabits when not feeding (figure 3). Mature caterpillars likely crawl down to the base of the plant to pupate in leaf litter (Pratt and Wiesenborn 2009).

Factors that influence sootywing life history include a restricted geographic range and high larval host plant specificity (Wiesenborn and Pratt 2008). Along with caterpillars being host specific, it has been suggested that only quailbush of a certain size and moisture content are suitable as sootywing oviposition substrates (Wiesenborn and Pratt 2008). It has also been implied that quailbush in areas

Figure 3.—Sootywing caterpillar inside its tent of leaves (from Cibola Valley Conservation Area-4-west-north in 2014).

near shade are preferred by sootywings (Pratt and Wiesenborn 2011) because sootywings are less physiologically adapted to sun exposure than some other sympatric butterflies (Wiesenborn 1999). Nectar plants such as alkali heliotrope (*Heliotropium curassavicum*) and sea purslane (*Sesuvium verrucosum*) are documented nectar sources suggested as important for maintaining sootywing populations, perhaps through increasing butterfly fecundity (Pratt and Wiesenborn 2011). Pratt and Wiesenborn (2011) indicate that dispersal between discontinuous and isolated population centers is unlikely, thus limiting a butterfly's ability to colonize new plots. It is unknown how far individual sootywings might move; however, Wiesenborn (1997) describes adults as easily traversing 13-foot (4-meter) spans across open terrain. Information from Wiesenborn and Pratt (2008) and Pratt and Wiesenborn (2011) show quailbush height, diameter, and plant lushness, along with soil moisture, shade, and nearby nectar sources, were important variables associated with sootywing presence. The occurrence of mesquite (*Prosopis* spp.) in the environment may also be notable since Wiesenborn (1997) suggests that mesquite extrafloral nectar (nectar source outside of flowers) may provide resources to sootywings when flowers are uncommon.

BUTTERFLY AND HABITAT MONITORING PROTOCOL DEVELOPMENT

Obtaining reliable population size estimates for butterflies is time consuming and expensive. In some cases, population estimates may be obtained through capture, mark, recapture techniques (Gall 1985). However, Murphy (1988) has made the case that handling of butterflies has adverse effects and recommends against using these methods for population studies with small-winged species (such as sootywings). In fiscal year 2014, the Bureau of Reclamation's (Reclamation) Technical Service Center began identifying monitoring methods that the LCR MSCP could use to monitor for presence of sootywings on LCR MSCP conservation areas.

Existing methods and literature were reviewed to identify sootywing habitat. The initial requirement for sootywing site selection was identified as presence of quailbush (Wiesenborn and Pratt 2008). Quailbush habitat on LCR MSCP conservation areas and previously occupied areas along the LCR were identified through onsite reconnaissance (Pratt and Wiesenborn 2011). The presence of quailbush alone does not ensure the presence of sootywings. Prior studies indicate that quailbush characteristics may also be important (Wiesenborn and Pratt 2008). Measures of quailbush characteristics were collected during sootywing presence surveys. Other estimates of sootywing habitat quality were also obtained: floral (nectar) metrics, soil moisture, and mesquite presence.

Windspeed (miles per hour [mph]) and air temperature (degrees Fahrenheit [°F]) were collected at the start of each sampling occasion and intermittently throughout sampling when wind and temperature changes were felt because of potential effects to butterfly detectability. Relative humidity (RH) measurements were also taken with a hand-held meter.

Information was collected on five quailbush at each patch of quailbush habitat surveyed. Patches ranged in size (see descriptions in table 1). A point count method was used. Observers would walk 5 minutes through a plot and then stop and record observations at the closest quailbush. The transects were not predetermined in order to randomly sample the plot, and transects were not linear because the path through the environment was guided by the density of quailbush and the presence of nectar plants. The transects differed each time a patch was surveyed. After environmental and shrub measurements were taken, surveyors looked for adult sootywings, eggs, and caterpillars on the selected shrub for 5 minutes. Then they would resume walking through the plot and repeat the process until characteristics of five quailbush were recorded.

Quailbush were measured for height and width. A rough qualitative estimate of percentage of dry or absent leaves was made as an indicator of plant lushness (in intervals of 5% or greater). Studies in semiarid grassland (Harrington 1991) showed that visual signs of water stress are good predictors of actual water stress and survival in woody plants. Soil moisture at each plant base was also measured (% saturation relative to field capacity; Kelway soil moisture tester Model HB-2). Lux was measured near the base of each plant to obtain some measure of shading provided by quailbush using a hand-held Extech 401025 light probe meter (resolution of 1 lux with 5% accuracy). RH measurements were also taken at each sampling point with a hand-held Extech Easy View 20 Hydro-Thermometer (RH range 10 to 95% with 0.1% resolution and basic accuracy of ±3% [30 to 95% RH] and ±5% [105 to 30% RH]).

Two people spent 5 minutes searching each selected quailbush for adult sootywings, eggs, and caterpillars. The numbers of each life stage observed were recorded. The presence of caterpillars provides evidence of reproduction in a given area, and Pratt and Wiesenborn (2011) also found site occupation by sootywings was most consistently identified by egg presence. Behaviors of detected adult sootywings were recorded as flying, perching, basking (wings open), nectaring (probing of flower with proboscis), puddling, mating, and ovipositing. A sootywing's sex was recorded when distinguished; sootywings were identified as females if they had paler and more mottled forewings and a larger body size. Other butterflies were also noted in the area as well as quailbush insect damage not associated with sootywings.

Floral abundance by plant species in the immediate environment was qualitatively noted, where 0 = none, 1 = scarce (flowers rarely encountered), 3 = common (flowers often observed), and 5 = abundant (floral abundance unlikely to be limiting). The floral index consisted of the sum of the value recorded for each plant species: if three separate plant species all had abundant flowers, the plot score would be 15. If only one plant species was observed and had abundant flowers, the plot score would be 5. This index favors both floral abundance and diversity of flower sources.

If, during a survey, eggs or caterpillars were found at additional quailbush, data on the location, adult behavior, temperature, and relative humidity were collected at these supplementary plants. Data were entered on a monitoring form (attachment 1).

Time Needed for Detection

Critical to a robust surveying program is an estimate of time needed for detection. Measures of time to detect sootywings (either eggs, caterpillars, or adults) were collected at Palo Ecological Reserve (PVER)-4, a plot where high numbers of adult sootywings were observed in 2013.

The time of day when sootywings were detected was also collected, along with an estimate of effort, to allow for determination of appropriate sampling periods. In some cases, organisms may exhibit diel periodicity in their activity patterns. Characterization of this pattern is important for determining sampling effectiveness.

Effort was defined as the proportion of a given hour sampled during different time intervals (e.g., 7–8, 8–9, 9–10….17–18, 19–20) throughout the day when monitoring occurred. If monitoring occurred from 7:30–8:45, then the 7–8 period would have an effort of 0.5, and the 8–9 period would score 0.75.

Monitoring Plots

Eight plots were selected from near Laughlin, Nevada, downstream to the Cibola NWR (table 1; attachment 2). The Cibola plot and the Hart Mine Marsh (HMM) plot are located in the Cibola NWR. Four of the plots were located in LCR MSCP conservation areas and included PVER-4, PVER-6-north, the Cibola Valley Conservation Area (CVCA)-4-west-north, and HMM. Plot names in the text are often shortened versions of the names in table 1. Plots ranged from xeric to mesic and included a range of quailbush sizes. Quailbush structure also varied widely at plots. Some plots contained quailbush that had small densely packed leaves with stunted plants, while others contained bushes with alternate growth forms. Quailbush at PVER-6-north differed from others in being low and broad in stature and infested with mildew, perhaps in response to standing water often observed at this plot. Groundcover also differed between plots, with some containing dense foliage and others barren (table 1).

Statistics

Preliminary analysis used a 2-way analysis of variance for statistical comparisons (where $\alpha = 0.05$) of quailbush characteristics ($n = 5$ plants) among plots and over months. Reclamation decided that formal statistical tests were not required for informing potential management actions; therefore, the results in this report include only summary statistics of quailbush parameters by plot (or plot and month where differences among plots appear to vary over time).

Table 1.—Plots used for sootywing monitoring along the LCR in 2014
(Plots are arranged from upstream to the furthest downstream plot.)

Plot name	Global Positioning System		Quailbush structure	Comments	Mesquite presence
	Northing	Easting			
Davis Dam SE (NW)	717372	3889366	Mostly dispersed plants; some densely packed. Bands of quailbush present.	Roads through area. Site bounded by roads and river. Isolated by river and development.	Northeast side of plot has fair number of mesquite.
Needles (NW)	720272	3857283	Patches of quailbush intermixed with arrowweed (*Pluchea sericea*).	River along one side. Boat launch area is along a portion of the plot.	Mesquite present.
PVER-4	730533	3730880	Rows of quailbush, often grown together.	Site bounded by roads and irrigation ditches. Thick Bermudagrass (*Cynodon dactylon*) groundcover. Plot irrigated.	Mesquite is common.
PVER-6-north	731007	3732414	Most quailbush appear stunted, with very small, densely packed leaves.	Mildew very common on quailbush leaves. Grasses growing up through quailbush. Plot irrigated.	Mesquite present.
Blythe NE (NE)	728265	3730648	Leaves tend to be very small on quailbush on side toward road. Very dispersed.	Surrounded by road and irrigation ditch. No groundcover.	Mesquite present.
CVCA-4-west-north	714200	3698916	Planted in rows interspersed with mesquite.	Roads demarcate plot. Edge of plot is moist because of leaky canal gates.	Mesquite present.
Cibola	714387	3687217	Plants very dispersed.	Road and mesquite on edges. No groundcover.	Mesquite present.
HMM	717893	3685758	Plants dispersed. Many plants dome-like in shape.	Lots of burro (*Equus africanus asinus*) use in area. Isolated patch of quailbush. High groundwater.	No mesquite.

Differences in four characteristics of plants "used" and "unused" by early life stages of sootywings were tested with *t*-tests. The Bonferroni correction was applied to the suite of tests for each characteristic to control α at 0.05 (comparisons were considered significant if $p \leq 0.0125$). A Pearson correlation was employed to test for relationships between variables and sootywing abundance. Data were normalized with a (ln (X+1)) or arcsin square root transformation for percentage data if the Shapiro-Wilks Test indicated non-normality.

Box and whisker plots were used to graphically compare environmental variables at plots. Boxes enclose the middle half of the data and are bisected by a line that represents the median value. Vertical lines at the box top and bottom represent the range of typical data values. Asterisks represent possible outliers, and o's represent probable outliers. Possible outliers are outside the box boundaries by greater than 1.5 times the box size, and probable outliers are outside the boundaries by more than 3 times the box size. The software Statistix 8 was used for performing the analyses. Graphs were created with Microsoft Excel, Statistix 8, and package ggplot2 (version 2.2.1) in Microsoft R Open (version 3.4.4).

Environmental DNA

Reclamation utilized funding from the Science and Technology Program to investigate the feasibility of using environmental DNA to detect the presence of sootywings. Sootywing samples were analyzed using universal DNA primers to obtain an initial mitochondrial gene cytochrome oxidase I (COI) sequence used to design sootywing-specific polymerase chain reaction (PCR) primers. Following the DNA sequencing of the COI gene, the sootywing samples were aligned and primers specific to the organism were designed using IDT DNA Primer Quest. Primer set, SW1, gives a 200 base pair (bp) PCR product. Primer set, SW2, gives a 210-bp PCR product.

RESULTS

Environmental variables

Windspeed averaged 2 mph (3 kilometers per hour) and ranged from 0–8 mph (0–13 kilometers per hour). This equates to most values being less than a light breeze on the Beaufort scale (an empirical measure of wind strength ranging from calm [force 0] to hurricane [force 12]). Temperature and RH from 4 months of sampling are presented on figure 4. Typically, temperatures increased in early to late afternoon and started declining around 16:00. RH was high in the mornings, declined somewhat at mid-day, and then increased toward the evening (figure 4).

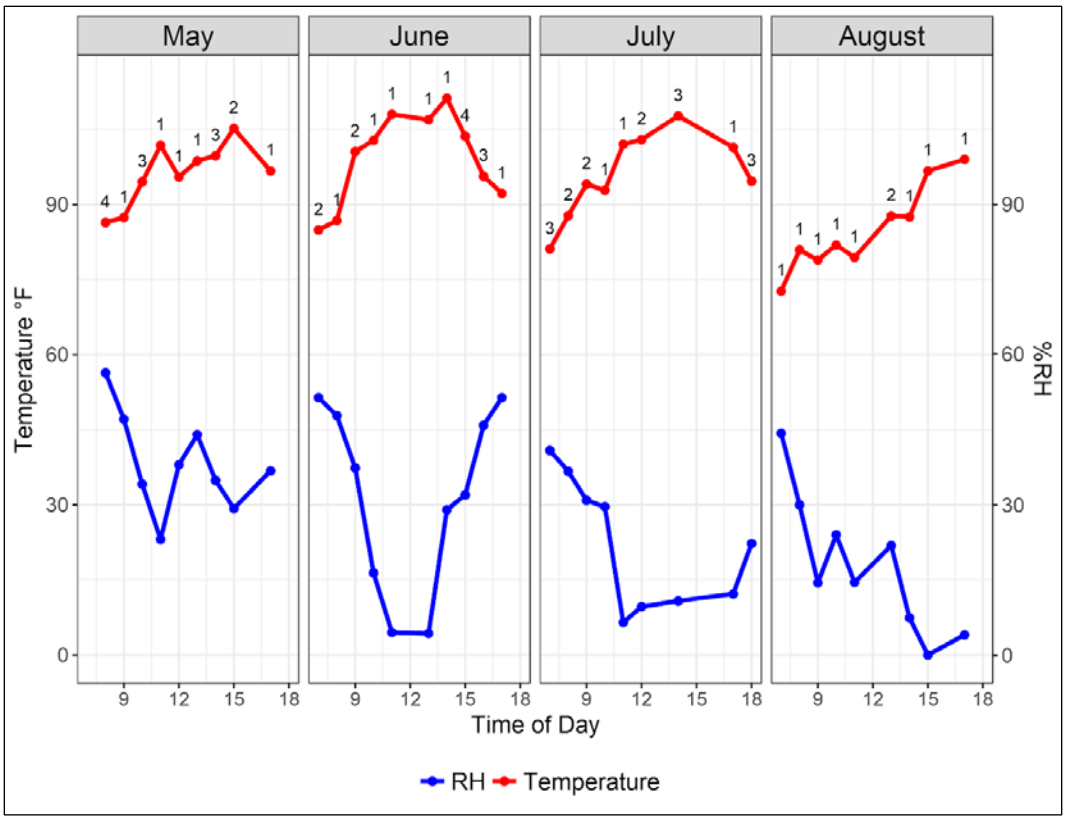

Figure 4.—Mean temperature and RH by time (hour) of day for each month using data from all eight monitoring sites.
The number of observations for each mean are noted above temperature trends.

Differences in soil moisture among sites varied over months (table 2). In May, HMM (mean $\bar{X}$ = 70 %), PVER-6-north ($\bar{X}$ = 62%), and PVER-4 ($\bar{X}$ = 68.4%) had high soil moisture relative to the other plots (range of mean values 0–16%). Soil moisture remained much higher in PVER-4 and PVER-6-north in June and July ($\bar{X}$ = 75–100% versus approximately 40% at HMM and 0–20% at other sites). PVER-4 and PVER-6-north are both irrigated, and the HMM plot is next to the marsh, which maintains high groundwater levels. Monsoonal rains just prior to the sampling in August 2014 resulted in higher soil moisture readings throughout the area (table 2). Needles, California, recorded 2.22 inches (56 millimeters) of precipitation in August, 560% of normal. The summer of 2014 was the wettest in Needles'67-year history, with 4.43 inches (113 millimeters) of total precipitation (National Oceanic and Atmospheric Administration National Climatic Data Center 2014).

Quailbush Characteristics

Summary statistics of quailbush characteristics are presented in table 3. Quailbush heights at PVER-6-north, CVCA-4-west-north, and the Cibola plot

Table 2.—Summary statistics for percent soil moisture by site and month (n = 5, except for David Dam in May [n = 3]).

Month	Plot name	Mean	Range	Standard deviation	Standard error
May	Davis Dam SE (NW)	0	0–0	0	0
	Needles (NW)	0	0–0	0	0
	PVER-4	68.4	30–92	23.3	10.4
	PVER-6-north	62	55–70	5.7	2.5
	Blythe NE (NE)	0	0–0	0	0
	CVCA-4-west-north	0	0–0	0	0
	Cibola	16	0–40	15.2	6.8
	HMM	70	20–100	41.2	18.4
June	Davis Dam SE (NW)	0	0–0	0	0
	Needles (NW)	0	0–0	0	0
	PVER-4	96	90–100	5.5	2.4
	PVER-6-north	100	100–100	0	0
	Blythe NE (NE)	0	0–0	0	0
	CVCA-4-west-north	20	0–100	44.7	20.0
	Cibola	0	0–0	0	0
	HMM	37	10–95	33.5	15.0
July	Davis Dam SE (NW)	0	0–0	0	0
	Needles (NW)	1.4	0–5	2.2	1.0
	PVER-4	100	100–100	0	0
	PVER-6-north	75	35–100	26.9	12.0
	Blythe NE (NE)	0	0–0	0	0
	CVCA-4-west-north	1	0–5	2.2	1.0
	Cibola	0	0–0	0	0
	HMM	42	5–80	28.0	12.5
August	Davis Dam SE (NW)	74	10–100	37.1	16.6
	Needles (NW)	21	5–50	18.8	8.4
	PVER-4	100	100–100	0	0
	PVER-6-north	100	100–100	0	0
	Blythe NE (NE)	62	50–90	16.4	7.3
	CVCA-4-west-north	100	100–100	0	0
	Cibola	100	100–100	0	0
	HMM	100	100–100	0	0

Table 3.—Mean ± standard error for quailbush height and width
(Range of values is in parentheses.)

Plot name	Quailbush (n = 20)	
	Height (feet)	Width (feet)
Davis Dam SE (NW)	8.3 ± 0.9 (3.0 –16.0)	10.6 ± 1.0 (3.0 – 20.0)
Needles (NW)	8.5 ± 0.8 (4.0 – 18.0)	10.2 ± 0.9 (4.0 – 20.0)
PVER-4	10.2 ± 0.7 (5.0 – 17.0)	12.3 ± 0.8 (7.0 – 20.0)
PVER-6-north	4.2 ± 0.3 (2.5 – 7.0)	9.5 ± 0.6 (3.0 – 16.0)
Blythe NE (NE)	9.2 ± 0.7 (5.0 – 15.0)	11.6 ± 1.2 (6.0 – 22.0)
CVCA-4-west-north	5.1 ± 0.4 (2.0 – 8.0)	4.3 ± 0.5 (1.0 – 10.0)
Cibola	6.0 ± 0.3 (3.0 – 8.0)	9.6 ± 0.8 (4.0 – 16.0)
HMM	10.4 ± 0.6 (5.0 – 16.0)	14.0 ± 1.0 (5.0 – 24.0)

were lower than at most other plots (see table 3). The tallest plants occurred at PVER-4 and HMM (see table 3). The narrowest plants were at CVCA-4-west-north. Differences in quailbush dryness among sites varied over months, similar to soil moisture. While the highest mean dryness values were found at CVCA-4-west-north in every month, dryness decreased over the season, especially at sites receiving regular irrigation (table 4; e.g., PVER-4 and PVER-6-north). Percent dryness was generally the lowest in August, after the heavy monsoon rains; however, dryness substantially increased from July to August at PVER-6-north.

Plot averages for all months (n = 32) ranged from 48–86% shade provided by quailbush. There was a single recorded value of 1.8% shade that was considered an outlier.

Quailbush were examined for eggs and caterpillars. Characteristics of occupied plants were compared to mean values of other randomly selected quailbush (all eight plots) to identify characteristics potentially important to utilization for oviposition (eggs) and caterpillars. Plants were identified as "used" (n = 12) or "unused" (n = 160) by sootywings. It is unlikely the plant condition changed between sootywing oviposition and the discovery of eggs on a particular plant.

Table 4.—Mean, standard error, and range for quailbush percent dryness by plot and month 9 (n = 5)

Month	Plot name	Range	Mean	Standard error
May	Davis Dam SE (NW)	1–2	1.2	0.2
	Needles (NW)	7–25	15.4	3.3
	PVER-4	0–10	3.2	1.8
	PVER-6-north	25–50	32	4.6
	Blythe NE (NE)	1–50	16.8	8.8
	CVCA-4-west-north	10–90	41	15.0
	Cibola	0–75	20	13.9
	HMM	0–25	5.6	4.9
June	Davis Dam SE (NW)	0.5–10	3.8	1.8
	Needles (NW)	0–75	19.1	14.2
	PVER-4	4–17	9.4	2.2
	PVER-6-north	3–16	10.2	2.1
	Blythe NE (NE)	4–70	33.8	11.9
	CVCA-4-west-north	1–80	46.2	13.1
	Cibola	0–50	15	8.9
	HMM	2–17	8.8	2.6
July	Davis Dam SE (NW)	2–35	20.4	7.2
	Needles (NW)	0–30	17.2	6.9
	PVER-4	1–7	3.6	1.1
	PVER-6-north	1–10	3	1.8
	Blythe NE (NE)	1–20	9.4	3.2
	CVCA-4-west-north	1–45	29.4	7.5
	Cibola	0–10	2.8	1.8
	HMM	3–10	5.8	1.4
August	Davis Dam SE (NW)	1–1	1	0.0
	Needles (NW)	0–30	7.4	5.7
	PVER-4	0–2	1	0.3
	PVER-6-north	10–30	17.4	4.2
	Blythe NE (NE)	0–4	1.4	0.7
	CVCA-4-west-north	2–40	22.4	7.1
	Cibola	1–2	1.2	0.2
	HMM	1–30	7.6	5.6

It is possible, however, that some plants contained undetected eggs and/or caterpillars and were placed in the incorrect category, decreasing robustness of this comparison. The small sample size for "used" plants also provides a reason to be cautious in interpreting results. Analyses (2-sample *t*-test) indicated no significant differences between used and unused quailbush for height ($p = 0.9284$) or width ($p = 0.4880$) (figure 5), nor for soil moisture (arcsin square root transformation) ($p = 0.5517$) at the plant base (figure 6a). The height of used quailbush ranged from 3–15 feet (0.9–4.6 meters), while the width ranged from 3 to 25 feet (0.9–7.6 meters). The median value for soil moisture at plants used by sootywings was much higher than in plants identified as unused (65 versus 25%, figure 6a), primarily due to the skewed distribution for unused plants (many 0 values). Mean values were more similar (50 versus 43%). The *t*-test for comparison of the percent dry vegetation (a measure of plant lushness) between used and unused plants was significant ($p < 0.0001$, arcsin squar eroot transformed data) (figure 6b).

Sootywing eggs and caterpillars were found on lush quailbush with lower amounts of dry vegetation. Values for occupied plants ranged from 0–12% dry (average 3% dry, back-transformed mean 1.8%). Unoccupied plants averaged 13% dry (back-transformed mean 9.3%) and ranged as high as 90% dry.

Eggs and caterpillars were found at heights from 1.5 to 7.0 feet (0.5 to 2.1 meters ($\bar{X}$ = 3.6 feet, 1.1 meters) from the ground in quailbush. Quailbush at PVER-6-north were anomalous to most other plots. In many cases, quailbush vegetation was recorded as being very dry (25–50% dry in May) despite high levels of soil moisture (50–70%). Many of these leaves appeared to be dying, perhaps because of excessive water (standing water was often present at this plot). In some cases, leaves were covered with mildew and described as chlorotic. Interestingly, when sootywings were finally detected in July at this plot, quailbush leaves were recorded as lush (1–10% dry), mildew was absent, and leaves on some examined quailbush appeared normal.

Floral Index

The floral index was similar among months, although the maximum observed value decreased from May to August (figure 7). The highest values were observed at PVER-6-north (figure 8). Saltcedar (*Tamarix* spp.), alfalfa (*Medicago sativa*), mesquite, and arrowweed (*Pluchea sericea*) appeared to be the most common sources of nectar. Alkali heliotrope and sea purslane, considered important nectar plants for sootywings, were rarely encountered. Overall, the floral index suggested low diversity and amounts of flowers and nectar in the environment for most months and plots, with few plant species recorded as abundant at plots.

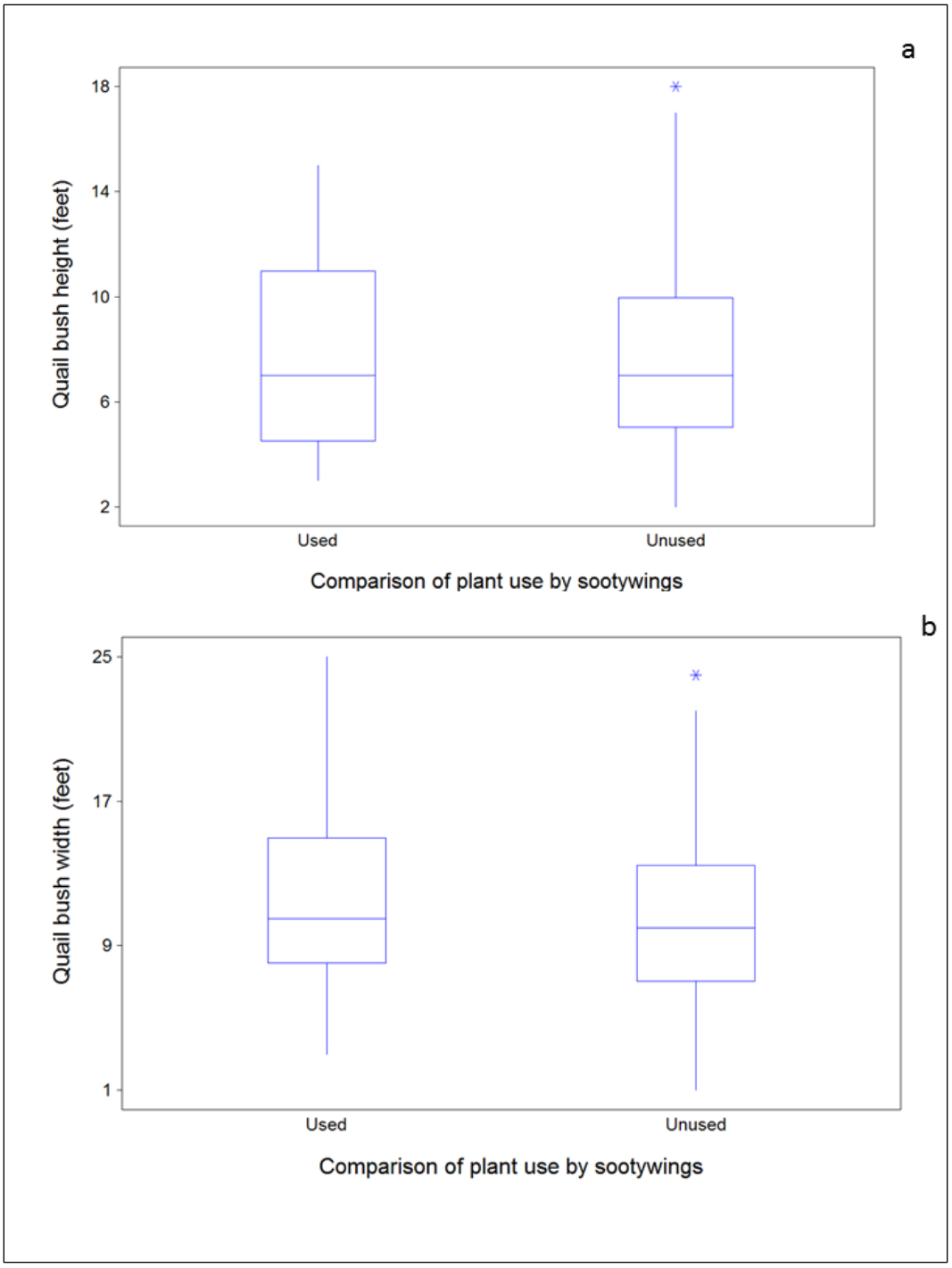

Figure 5.—Box plots comparing quailbush height (a) and width (b) between shrubs used by sootywings for egg oviposition/caterpillars and those where early life stages were not detected.
No significant differences were detected between used and unused plants.

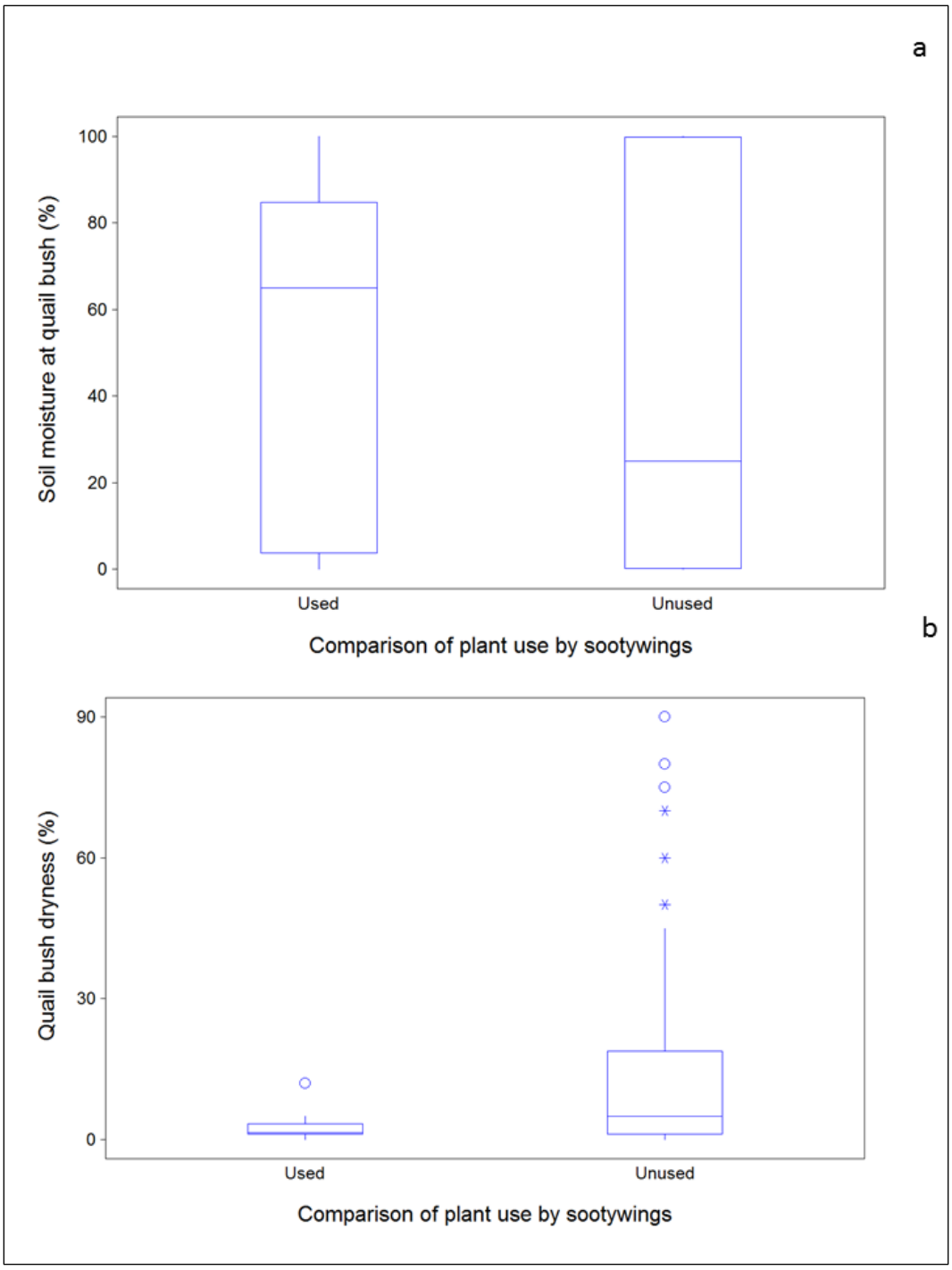

Figure 6.—Box plots comparing quailbush soil moisture (a) and percent dryness (b) between shrubs used by sootywings for egg oviposition and by caterpillars and those on which early life stages were not detected.
A significant difference was detected in percent dry vegetation at used and unused shrubs (b).

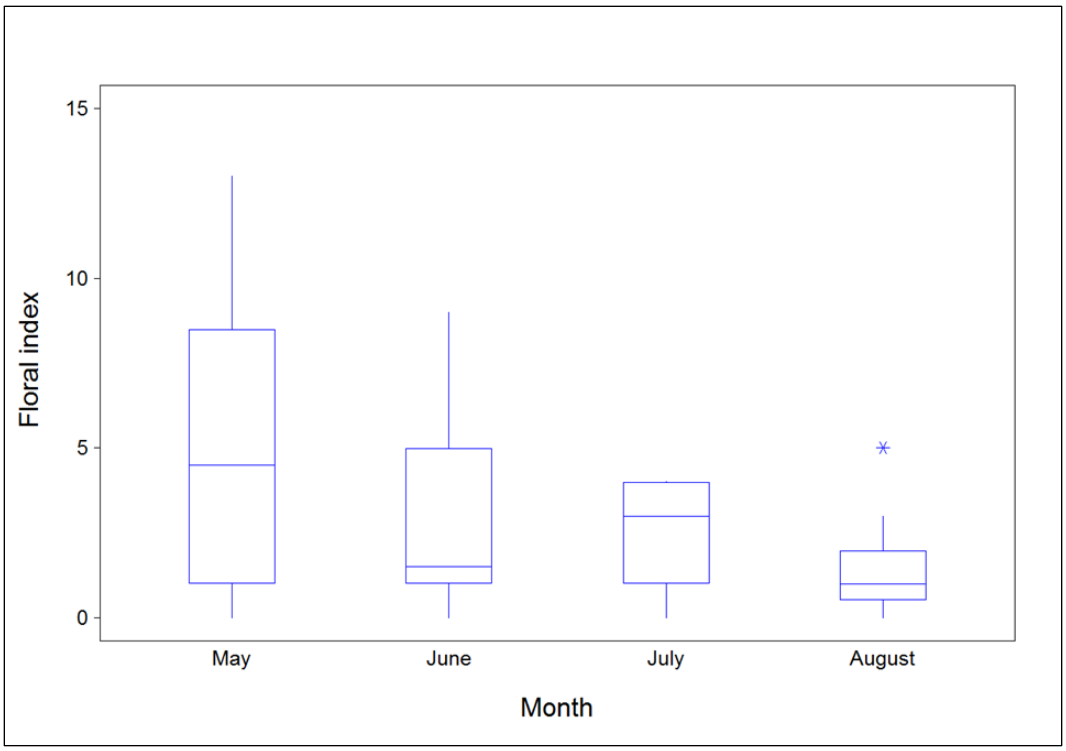

Figure 7.—Box plot of the floral index by month from plot data along the LCR (n = 8).

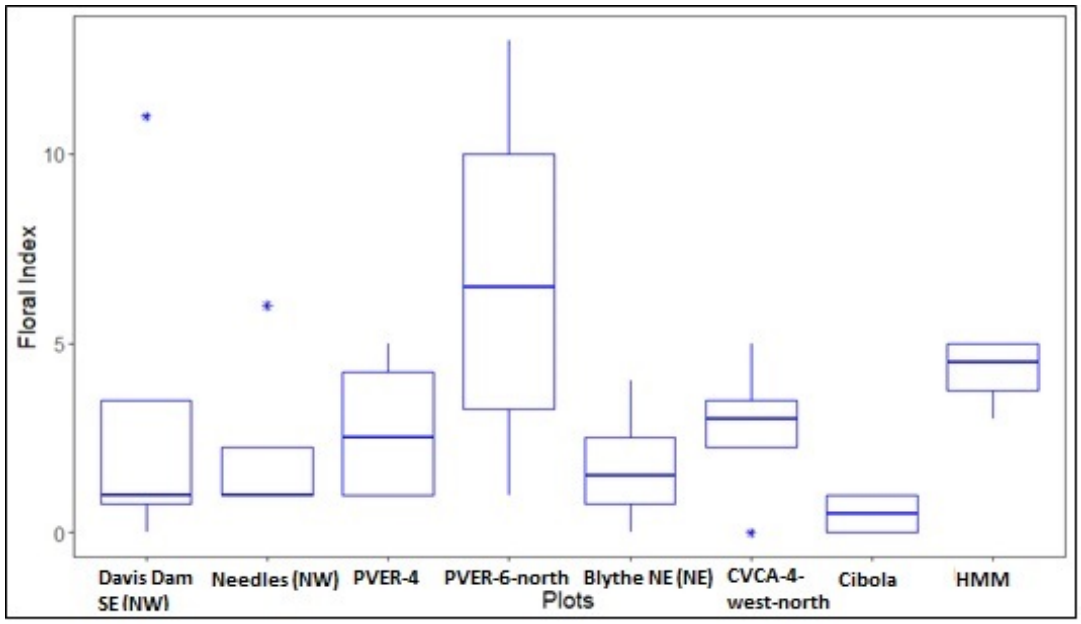

Figure 8.—Box plot of the floral index by plot from data along the LCR (n = 4).

Nectaring activity was only observed in 4 individual sootywings out of 49 noted behaviors. Two individuals were observed nectaring at alfalfa, while in August, two individuals were observed nectaring at quailbush. Quailbush, to our knowledge, has not previously been reported as a nectar plant for sootywings. Saltbush species (*Atriplex* spp.) are believed to be mostly wind pollinated (Blackwell and Powell 1981); however, evidence clearly demonstrating this for quailbush is lacking (Meyer 2005). Quailbush were not included in the qualitative floral index for this reason, but perhaps the shrub should be acknowledged during flowering periods in future surveys. It may also be of value to examine quailbush flowers for sugar content. Sootywing abundance was not significantly correlated with the floral index ($p \geq 0.27$). The floral index was correlated with soil moisture ($r = 0.4568$, $p = 0.0248$), but only if the month of August (moist soil because of recent precipitation) was omitted. Sampling in August likely occurred soon enough after precipitation events that nectar resources did not have time to respond to the increased moisture.

Sootywing Presence

Sootywings were detected at all eight plots (table 5). Adults were detected at seven plots (if the revisit to Needles (NW) is included), eggs at four plots, and larvae at five plots. The only plot at which sootywings were detected in every month was PVER-4. Sootywings were detected during three months at CVCA-4-west-north, the Cibola plot, and HMM, and during two months at PVER-6-north and Blythe NE (NE). Detection at Davis Dam SE (NW) and Needles (NW) occurred during 1 month. All three life stages were detected at three plots (PVER-4, PVER-6-north, and the Cibola plot). Only a single egg was detected at Davis Dam SE (NW). Adults were detected at Needles (NW), but only during an additional visit. Four out of eight plots were positive for sootywings in May; in June, two out of eight plots; in July, five out of eight plots; and in August, eight out of eight plots were positive for sootywings. Forty-two adult sootywings and 14 eggs/ caterpillars were detected during standard sampling (additional detections occurred during plot revisits). The ability to detect adult sootywings varied by month, with 8 individuals detected in May, 1 in June, 12 in July, and 21 in August (table 5). Increased soil moisture from precipitation events in August (see table 2) and corresponding increased quailbush lushness (decrease in dry vegetation) (see table 4) may have played a role in increased detection of adult sootywings.

Adult sootywing abundance was highest at PVER-4 and lowest at the furthest upstream plots at Davis Dam SE (NW) and Needles (figure 9). Abundance was lowest in June and highest in August. Data presented on figure 9 are from initial samplings and do not include any revisits to plots.

Adult sootywing abundance and the sum of adult sootywing and early life stages abundance (ln (X+1)) were correlated with quailbush dryness and soil moisture

Table 5.—Sootywing presence detections (X) on survey plots

(Sootywing adults were detected at seven plots (including Needles (NW) revisit), eggs at four plots, and larvae at five plots. Sootywings were detected at PVER-4 in all four months. Sootywings at CVCA-4-west-north, the Cibola plot, and HMM were detected in three months, while detections at PVER-6-north and Blythe NE (NE) occurred in two months. Sootywings at Davis Dam SE (NW) and Needles (NW) were only detected in a single month. All three life stages were observed at three sites (PVER-4, PVER-6-north, and the Cibola plot). At Davis Dam SE (NW), only a single egg was detected; at Needles (NW), adults were detected, but this was during a revisit(*). In May, four out of eight plots were positive for sootywings; in June, two out of eight plots; in July, five out of eight plots, and in August, eight out of eight plots were positive for sootywings.)

Plot name	Detections											
	May			June			July			August		
	Egg	Larvae	Adult	Egg	Larvae	Adult	Egg	Larvae	Adult	Egg	Larvae	Adult
Davis Dam SE (NW)	–	–	–	–	–	–	–	–	–	X	–	–
Needles (NW)	–	–	–	–	–	–	–	–	–	–	–	X*
PVER-4	X	X	X	–	–	X	–	–	X	–	–	X
PVER-6-north	–	–	–	–	–	–	X	X	X	X	–	X
Blythe NE (NE)	–	–	X	–	–	–	–	–	–	–	–	X
CVCA-4-west-north	–	–	–	–	X	–	–	X	X	–	–	X
Cibola	–	–	X	–	–	–	X	X	–	X	–	X
HMM	–	–	X	–	–	–	–	X	–	–	–	X
Total number detected	**1**	**1**	**8**	**0**	**1**	**1**	**5**	**4**	**12**	**3**	**0**	**21**

(both variables arcsin square root transformation). Adult sootywing abundance and summed values of adult and early life stages abundance were both negatively correlated with plant dryness (r = -0.3499, p = 0.0496; r = -0.4064, p = 0.0210) and positively correlated with soil moisture (r = 0.5740, p = 0.0006; r = 0.5282, p = 0.0019). Soil moisture and percent dry quailbush were negatively correlated with each other (r = -0.3578, p = 0.0444). Sootywing abundance values were not significantly correlated with quailbush height, width, or the floral metric ($p \geq 0.3$).

Forty-nine incidences of behavior were noted during the study. Because these were from visual observations, noted behaviors were likely skewed toward flying, which is probably the most easily detected behavior. The most common adult sootywing behavior was flying at 61% of the time, followed by perching/basking at 28%. Oviposition was observed on a single occasion (2% of behaviors) at 10:30 in the morning at the Cibola plot. Nectaring was observed in four cases (8% of behavior observations); most of these nectaring observations were relatively brief. However, in one case, an adult female was observed actively nectaring at an alfalfa plant for 11 minutes.

Time Needed for Detection and When Detected

The amount of time required and the preferred time of day for detection of adult sootywings was determined by repeat visits to PVER-4, a known sootywing site (table 6).

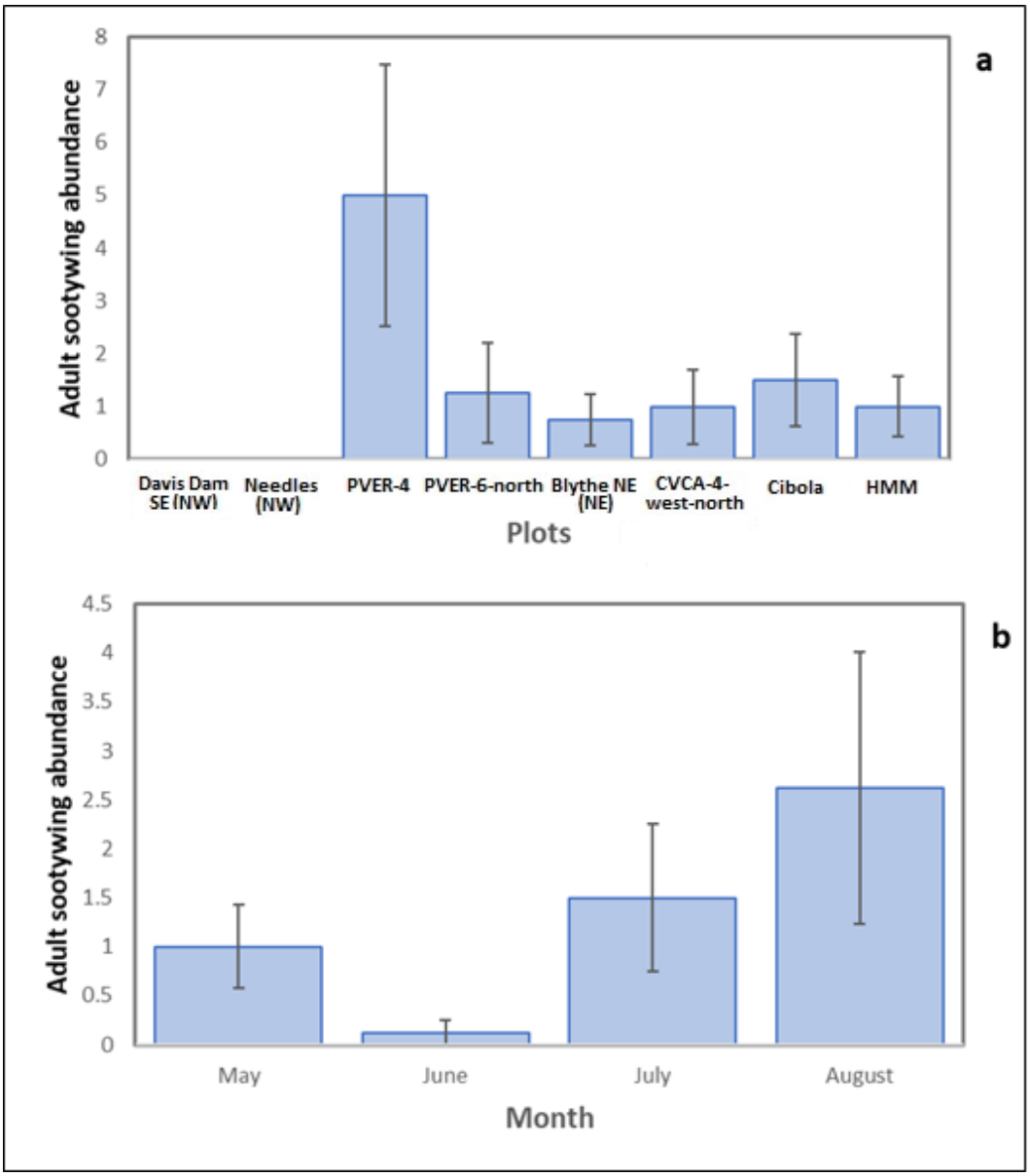

Figure 9.—Adult sootywing abundance (mean ± standard error) at plots (a) along the LCR (n = 4) and during months (b) in 2014 (n = 8).

This portion of the study was limited to PVER-4 because the site was relatively productive in 2013 and other plots may not have contained sootywings. Several samplings occurred during each month of the study at different times of the day.

The mean time and standard error to detect adult sootywings at PVER-4 was 32 ± 8 minutes (n = 13). Ninety-two percent of positive detections occurred within 1 hour (table 6). However, on four occasions, sootywings at PVER-4 were not detected within the sampled period of time. Of these, one sample period only lasted 37 minutes, perhaps indicating that not enough time had expired.

However, during sampling earlier the same day, 12 sootywings were detected, which was the high count for the year. The non-detection period occurred from 14:25 to 15:02. The other three non-detection samples ranged from 57 minutes to 1 hour and 25 minutes in time expended. Two of these were in late afternoon (14:14–15:11 and 14:55–16:20), while the other was relatively early in the day (7:12–8:35). These data suggest it might be wise to avoid late afternoon monitoring of adults; however, it should be acknowledged that on two other occasions, sootywings at PVER-4 were detected in the 14:00–15:00 time period. The time of sootywing detections at PVER-4 ranged from 8:47 to 18:12 (n = 13). The earliest observation, when all plots were included, was 7:41 at PVER-6-north in July.

Table 6.—Frequency distribution of time to detection of adult sootywings at PVER-4 (Highlighted information indicates the time wherein a high percentage of positive detections had occurred. Ninety-two percent of all detections took place within 51 minutes.)

Time to detection (minutes)	Frequency	Percent	Cumulative	
			Frequency	Percent
1	1	7.7	1	7.7
5	1	7.7	2	15.4
15	1	7.7	3	23.1
21	2	15.4	5	38.5
22	1	7.7	6	46.2
24	1	7.7	7	53.9
25	1	7.7	8	61.6
32	1	7.7	9	69.3
39	2	15.4	11	84.7
51	1	7.7	12	92.4
119	1	7.7	13	100.1
Total	**13**	**100**		

As an additional test of adult sootywing detectability relative to time of day, detections per unit effort were examined for all eight plots. These results also suggest mornings are best for detection, with a lull in the ability to detect sootywings in the late afternoon (figure 10). A fair amount of detection effort occurred in the late afternoon (figure 10), but few sootywings were seen. This supports conclusions from data limited to PVER-4. Plots sampled in the late afternoon between 14:00–16:00 included PVER-4, PVER-6-north, Davis Dam SE (NW), and Needles (NW). This lull in activity may be a direct response to increased air temperatures, which occurred around this time of day (see figure 4). The amount of effort was not uniform for all time intervals, and it may be important to collect further data to demonstrate this in a more robust manner.

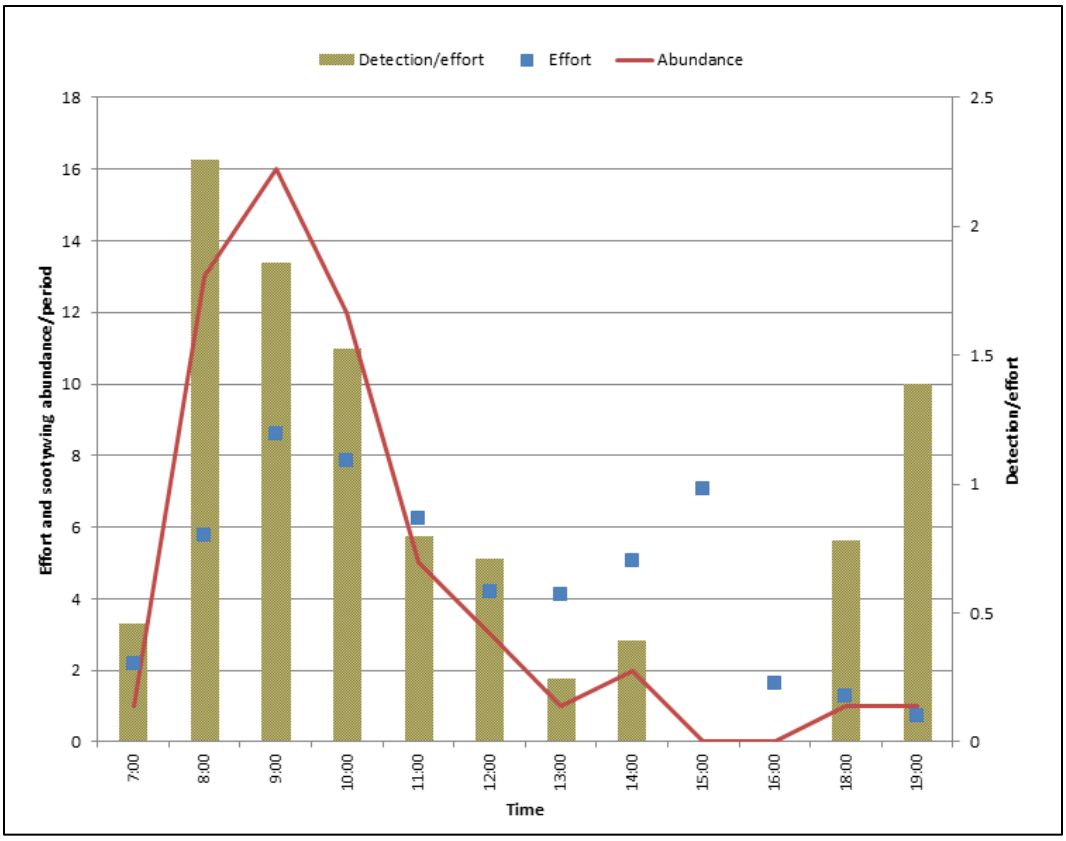

Figure 10.—Sootywing detections per unit effort (shown as bars).
Also shown is the amount of effort expended and the number of adult sootywings counted in each time period.

Because of travel considerations, the two furthest upriver plots were consistently sampled in early and late afternoon. Sootywings were undetected at these plots until August, when a single egg was found at Davis Dam SE (NW). If late afternoon corresponds to a lull in sootywing activity, it is possible that adult sootywings present could have avoided detection at these two plots because of timing. The upriver Needles (NW) plot was revisited at 10:50 in the morning on the final sampling week in August, following the late afternoon sampling earlier in the week. Within 15 minutes, two adult sootywings were detected. However, recent rains, which occurred in August, may have played some role in the ability to detect sootywings at these plots that had limited soil moisture (see figure 6) in most months.

Other Invertebrates

Other butterflies observed at monitored plots included pygmy blue (*Brephidium exilis*), marine blue (*Leptotes marina*), ceraunus blue (*Hemiargus ceraunus*), Reakirt's blue (*Echinargus isola*), checkered white (*Pontia protodice*),

orange sulfur (*Colias eurytheme*), dainty sulfur (*Nathalis iole*), common checkered-skipper (*Pyrgus communis*), eufala skipper (*Lerodea eufala*), and fiery skipper (*Hylephila phyleus*). Some of these species were likely using plot vegetation. Marine, ceraunus, and Reakirt's blues use mesquite as a larval host plant, and a pygmy blue was observed laying eggs on quailbush seed heads. Eufala skippers were relatively common at some plots that had Bermudagrass (*Cynodon dactylon*) (a caterpillar host plant) as groundcover.

Additional taxa utilizing quailbush included Ensign coccids (Ortheziidae), aphids (Aphidoidea), galls (various insects), grasshoppers (Orthoptera), cicadas (*Diceroprocta apache*) (which caused egg-laying damage), and the moth *Trichocosmia inornata*. *Trichocosmia inornata* was identified by DNA analysis from a caterpillar found feeding on quailbush. Ensign coccids were especially common at the Needles (NW) plot, where they appeared to impact quailbush health to some degree.

Amount of Habitat Needed for Sootywings

The 28-acre CVCA-4-west-north plot provided an example of the importance of water and the presumed minimal area needed for sootywing persistence. The area sampled in 2014 had been planted with quailbush and mesquite and, in the past, when irrigation was more frequent, had supported sootywings (Nelson et al. 2014). Irrigation has recently been less frequent, and much of the quailbush appeared highly stressed, with only a single sootywing reported from the plot in 2013 (Nelson et al. 2014). While most of the plot in 2014 appeared, because of the poor condition (mean soil moisture = 0.4 $\pm$ 0.4%, n = 14, August excluded) of quailbush (in many cases touching a plant would result in leaf fall), to be devoid of sootywings, a narrow band of habitat was discovered along one edge that contained lush quailbush. Water from leaky canal gates off a main ditch provided moisture (mean soil moisture = 67 $\pm$ 33%; n = 3) for maintenance of high-quality quailbush, allowing for the presence of sootywings. Three quailbush in this small section of habitat were measured, and there were differences (Bonferroni correction would result in $\alpha = 0.0167$) in quailbush characteristics between the main plot and the small watered area (height, $F = 25.84$, $p = 0.0001$; width, $F = 38.96$, $p < 0.0001$; percent dry vegetation, $F = 8.03$, $p = 0.0102$) (main plot; height, $\bar{X} = 4.9 \pm 0.4$ feet (1.49 ± 0.12 meters), width, 4.0 ± 0.5 feet (1.22 ± 0.15 meters), percent dry vegetation, 36 ± 6%, n = 19) (small watered area; height $\bar{X} = 11.7 \pm 2$ feet (3.57 ± 0.61 meters), width 16.7 ± 4.6 feet (5.09 ± 1.40 meters), percent dry vegetation, 2.0 ± 1.0, n = 3). It appeared that a Fremont cottonwood-Goodding's willow (*Populus fremontii-Salix gooddingii*) plot further down the canal was being irrigated with some frequency and the charged canal was providing sufficient moisture for a small linear area (about 16,000 square feet, 0.00037 acre) of quailbush.

Of the 28 acres of quailbush and mesquite at the CVCA-4-west-north plot, it is suspected that < 0.00122% of the plot was supportive of sootywings. Both larvae and adults were detected at this small section of the plot (see table 5).

Environmental DNA

Following the DNA sequencing of the COI gene, sootywing samples were aligned, and primers specific to this organism were designed using IDT DNA Primer Quest. Primer set SW1 gives a 200-bp PCR product, and primer set SW2 gives a 210-bp PCR product. Primers were tested against known sootywing samples and a moth sample. The moth sample gave no PCR product, while both sootywing samples gave positive PCR results.

DISCUSSION

Quailbush/Environmental Characteristics

Sootywings were detected at all sites despite variable quailbush characteristics (plant height and width). Groundcover and the presence of mesquite also varied between occupied plots. Eggs or caterpillars were found on shrubs that ranged in width from 3–25 feet (0.9–7.6 meters). Shrub height or width of the small sample of occupied plants did not differ from those without sootywing eggs or larvae. A range of quailbush sizes may be desirable in plots managed for sootywings to provide enough resources for young larvae to reach the pupation stage, and large shrubs may be important to adults as shade sources. Shade for sootywings can be provided by quailbush; other shade plants, such as mesquite, may not be required. Plants occupied by eggs and larvae did differ in lushness between occupied and unoccupied shrubs, with occupied plants significantly less dry than those in which eggs or larvae were not detected. Pratt and Wiesenborn (2011) indicate sootywing presence is related more to host plant quality rather than host plant abundance. Herbivores, in general, and sootywings specifically, often seek out plants with higher moisture contents (Myers 1985; Grundel et al. 1998; Wiesenborn and Pratt 2008) and corresponding higher nitrogen content. Typically there is a positive correlation between nitrogen and plant water content (Mattson, Jr. 1980), and this has been found to be the case for quailbush (Wiesenborn and Pratt 2008). Increased environmental moisture may trigger new vegetation growth that is nitrogen rich and succulent. Nitrogen content of leaf matter is correlated with increased lepidopteran caterpillar growth rates (Coley et al. 2006). Higher leaf water content is also associated with increased rates of insect larval development (Myers 1985), while decreased environmental moisture induces low food conversion rates in Lepidoptera (Mattson, Jr. 1980). Myers (1985) suggests butterflies can recognize leaf water content through visual cues, such as color and size of plants, which indicate physiological status. This

plant status affects growth rates of caterpillars and survival to pupation (Myers 1985; Grundel et al. 1998). Wiesenborn and Pratt (2008) suggest that suitable high-quality quailbush is a limiting factor for sootywings along the LCR because of inadequate soil moisture throughout most of their range. Situations in which there is too much water may also occur (PVER-6-north), and it has been shown that waterlogging has major impacts on plant growth for many *Atriplex* species (Galloway and Davidson 1993), including quailbush (Parveen et al. 2002). Some *Atriplex* species may be able to recover rapidly from waterlogging conditions (Galloway and Davidson 1993), and this may correspond to the changes in plant suitability for sootywings observed at PVER-6-north between sampling months.

Floral metrics were not associated with sootywing abundance. Floral resources seemed limited in most months in 2014. The important nectar plants alkali heliotrope and sea purslane (Pratt and Wiesenborn 2011) were uncommon flower sources at the plots monitored. Alkali heliotrope flowers were recorded twice, once each in May and June. Sea purslane flowers were documented on a single occasion in May. Saltcedar was the flower source most commonly encountered and with highest floral abundance. Wiesenborn and Pratt (2010) consider sootywings generalist nectar feeders, and Scott (1986) indicates sootywings only nectar occasionally. Nectaring behavior was observed at alfalfa and quailbush (sootywings probing flowers with their proboscis). The assumption is that some nutritional value was associated with sootywings probing quailbush flowers. Wiesenborn and Pratt (2010) indicate that quailbush is a wind-pollinated shrub without nectar. However, bee foraging has been observed at flowers of quailbush in other areas (Morandin and Kremen 2013). It is possible that the noted bee foraging activity was for collection of pollen rather than nectar.

Pratt and Wiesenborn (2009) observed a large number of sootywing behaviors (1,620) and noted that 12% of these behaviors involved landing on flowers (nectaring), which might be considered similar to the 8% nectaring observed in this 2014 study. While Pratt and Wiesenborn (2011) indicated nectar plant abundance was important to increasing fecundity, the observations in 2014 seem to indicate that abundant nectar is not obligatory for sootywing presence at a plot. Butterfly species differ in their use of nectar. Loertscher et al. (1995) compared five different butterfly species and found species-specific nectaring behavior varying from 0.2 to 53% of observed behaviors. In a study of another LCR butterfly, the western viceroy (*Limenitis archippus obsoleta*), Nelson (2003) notes nectaring in 12.7% of cases; however, puddling (another method of obtaining moisture) was documented in 10.4% of observed behaviors. No evidence of puddling has been observed in sootywings.

High-quality quailbush may be especially important to sootywing survival if nectar sources are limited. The lifespan in adult butterflies can be affected by both larval host plant quality and adult nectar resources. Beck and Fiedler (2009) suggest that highly active nectar feeders may be compensating for poor larval host plant quality by obtaining adult foods rich in amino acids. Conversely,

high-quality larval host plants may make adult feeding less important for maintaining an adequate lifespan. Longer lifespans may be important for egg maturation in female butterflies (Beck and Fiedler 2009).

Potentially, the relatively low rate of flower visits could impact the possibility of detection of sootywing eDNA with attraction targets that mimic flowers and induce sootywing landings.

Sootywing Behavior

Flying (61%) was the most common activity and oviposition (2% of behaviors) the least common behavior observed. These observations are similar to those of Pratt and Wiesenborn's (2009) study in which flying (43% of observed behaviors) and oviposition (1% of observed behaviors) were also the most common and least common behaviors. Perching/basking was seen in 28% of behaviors, and nectaring was observed in 4 cases (8% of behavior observations). A lull in sootywing activity was also observed during the hottest time of the day; however, this observation was based on limited data.

Time Needed for Detection and When Detected

Detection bias can be reduced through appropriate timing of sampling. It seems likely that expending at least 1 hour for detection of adult sootywings at a plot is an appropriate amount of time and provides some balance between sampling effort and probability of detection when they are relatively common at a survey area. It should be recognized, however, that even at a relatively productive site, there are occasions when adults will not be detected. Repeat visits over several months will help ameliorate non-detections at occupied sites.

Adult sootywings may be difficult to detect when temperatures are highest during the day. The graph of temperature versus time of day (see figure 4) indicates that the lull in adult sootywing activity corresponds roughly with highest daily temperatures. Wiesenborn (1999) demonstrates that sootywings were quicker to seek shade at higher temperatures and provides a reason for the decreased detection during afternoon periods at plots along the LCR during 2014. Adult sootywings are likely immobile and perched inside shade-producing quailbush at the hottest time of the day. Other butterflies have also been reported to seek shade and cease active flight above certain temperatures (Clench 1966). Pratt and Wiesenborn (2009) indicate that sootywing surveys appear most effective during April – May and during the morning, when adults were most active. Nelson and

Andersen (1999) report sootywings as being commonly detected in March. Based on all these observations, sampling for adults is likely effective between 8:00 and 13:30 and then again after 16:00 until dusk.

Searching quailbush for early life stages may help in identifying occupied plots. During the surveys in 2014, it seemed that adults were the most easily detected life-stage; however, in some months, plots were identified as occupied with just eggs or caterpillars. Pratt and Wiesenborn (2011) found that egg and larvae detection identified more occupied sites than did presence of adult sootywings, but they also point out that detecting sootywing populations is most straight-forward through observation of adults (Pratt and Wiesenborn 2009).

Pratt and Wiesenborn (2009) also suggest that summer rainfalls may increase host plant water content and stimulate late season adult flights. Monitoring efforts in 2014 found that the highest number of sootywing detections occurred in August 2014, in conjunction with heavy precipitation just prior to sampling. Variation in precipitation as it affects host plant growth influences diapause in butterflies (Sims 1983). Extended diapause that occurs in late instar larvae or pupae is often observed in Lepidoptera that live in areas of seasonal drought (Powell 1987). Diapause is often broken after rains that provide moisture and encourage host plant growth (Mattoni et al. 1997). Some skippers found in areas with low and unpredictable rainfall aestivate for months as late instar larvae, with the adult flight season highly responsive to rainfall episodes (Palmer and Braby 2012). Sootywings may have similar strategies and responses to rainfall events along the LCR. These erratic population changes may require repeated sampling at some locations.

Sampling for a suitable length of time, at the appropriate time of day, and repeated sampling will increase detections of sootywings at quailbush plots and reduce detection bias.

Amount of Habitat Needed for Sootywings

Past papers have suggested that small amounts of habitat can successfully support sootywings. Pratt and Wiesenborn (2011) state, "Even relatively isolated patches of a few large, lush bushes can provide quality habitat for sootywings. Small numbers of quailbush can support populations of sootywings." Information from CVCA-4-west-north in this study also suggested that a small linear strip of moist habitat (16,000 square feet, 0.00037 acre) was responsible for sootywings detected in this area. Quailbush quality was much higher in this small area and suggests habitat areas may not need to be especially large to provide sootywing support. Maintenance of small core areas could allow for rapid expansion of sootywings into chronically less desirable areas that change in moisture level (and ability to support sootywings) from rains or modifications in irrigation.

Environmental DNA

Primers were developed and tested against known sootywing samples. With these primers it may be possible to analyze complex environmental samples for the existence of sootywing DNA that would indicate the presence or absence of this organism at a given location. Detection of sootywings from feeding-damaged leaves or from floral targets (although limited nectaring activity may make this infeasible) presented and landed upon in the environment may be achievable with these primers.

Development of eDNA techniques to determine presence at sites where no visual confirmation has occurred may be an important addition to the sootywing monitoring program. Techniques would need to be developed so that plant material DNA does not overwhelm sootywing DNA and therefore affect detection ability.

Because of difficulties in capture-mark-recapture studies and the complications in following individual sootywings through dense brush, migration metrics (dispersal + reproduction) and relationships between distances and isolated population centers and gene flow barriers may be best obtained through genetic studies (Black et al. 2001). These data may provide information on a sootywing's ability to colonize new plots.

Other

Repeated surveys at the same set of plots every year would result in a time series of sootywing presence that allow for estimation of changes in the population (presence/absence). Abundance trends in these cases would be inferred from changes in the number of sites within a region that switch status (sootywing presence or absence) between years. However, instead of repeatedly surveying a site after detection occurs in the first few months, it may be sensible to drop positive sites and add other additional plots. Dropping positive sites for a given year will allow for additional sampling to determine the geographic extent of populations. Additions may also be important if conditions at plots change over time to the point in which habitat is not suitable for sootywings. Sampling at new sites might allow for more accurate estimates of the condition of the regional population. Updated samples might, in some cases, be more representative of current populations. Perhaps a set of plots along the river examined in four separate months for presence/absence every year (specified set of 50 plots, with about 10 consistently monitored every year) might be appropriate. As soon as detection occurs at any of these 10, another plot is added, and the original plot is dropped. The number of sampled plots might also be a useful metric. During good sootywing years, numerous plots would be sampled, and during poor years, a smaller number would be monitored.

It may also be of some interest to collect information on quailbush that adult sootywings are using for perching and avoiding heat. A certain size plant may be obligatory for provision of appropriate cooling environments. It may also be of value to deploy temperature sensors in various-sized quailbush to determine whether temperature amelioration is plant-size dependent, and it would potentially provide evidence on the size of quailbush needed for adult temperature regulation.

Present and past studies show that measures of plant leaf health (dryness/nutrient status) are an important gage of sootywing habitat quality at quailbush plots, suggesting the importance of more quantitative measures of leaf/plant health. A more quantitative measure might provide an accurate endpoint of plant suitability for sootywing early life stages. A chlorophyll meter (Bullock and Anderson 1998) may present a good option for such measurements, as it rapidly measures chlorophyll content or "greenness" of plants. Indexed chlorophyll contents are obtained by clamping the meter over leafy tissues, and readings are obtained in a few seconds. Stefanescu et al. (2006) found *Euphydryas* butterflies selected leaves identified with a chlorophyll meter as being the "greenest" at oviposition sites.

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ATTACHMENT 1

MacNeill's Sootywing Skipper (*Pholisora gracielae* = *Hesperopsis gracielae* [MacNeill]) Monitoring Data Sheet

Date _____ Surveyor _____

Plot name _____

Global Positioning System _____

Time start _____ Time first detection _____ Time end _____

Air temperature (˚F) ____ % Relative Humidity ____ Windspeed (miles per hour) ____

Cloud cover _____

Open area initial lux (x100) _____ Final lux (x100) _____

Mesquite presence _____

Nectar by species (e.g., mesquite, arrowweed, heliotrope, alfalfa, etc.) in the environment. 0 = none, 1 = scarce, 3 = common, 5 = abundant, unlikely to be limiting.
_____

Overall quailbush structure (dispersed, densely packed, planted in rows) and leaf growth form (small versus large leaf area, leaf density)_____

Quailbush insect damage (**not** sootywing) and potential source of damage (e.g., grasshopper, aphid, Ensign coccids)
_____

Site bounded by roads, irrigation ditches, unsuitable habitat? Groundcover present?
_____

Other butterflies noted _____

Quailbush characteristics and presence of sootywing early life stages

Variables	Quailbush 1	Quailbush 2	Quailbush 3	Quailbush 4	Quailbush 5
Lux (x100) at plant base					
Quailbush height and width (feet)					
Quailbush percent dry					
Soil moisture (%) at base of quailbush					
Level of feeding damage consistent with sootywing caterpillars 1 – scarce to absent 3 – moderate, several branches impacted 5 – heavy, most-to-all branches with feeding					
Number of eggs					
Number of caterpillars					

Adult sootywing detections

Time	Sex	Adult behavior*

* Adult behaviors recorded as flying, perching, basking, nectaring, puddling, mating, and ovipositing.

ATTACHMENT 2

Maps of Study Areas

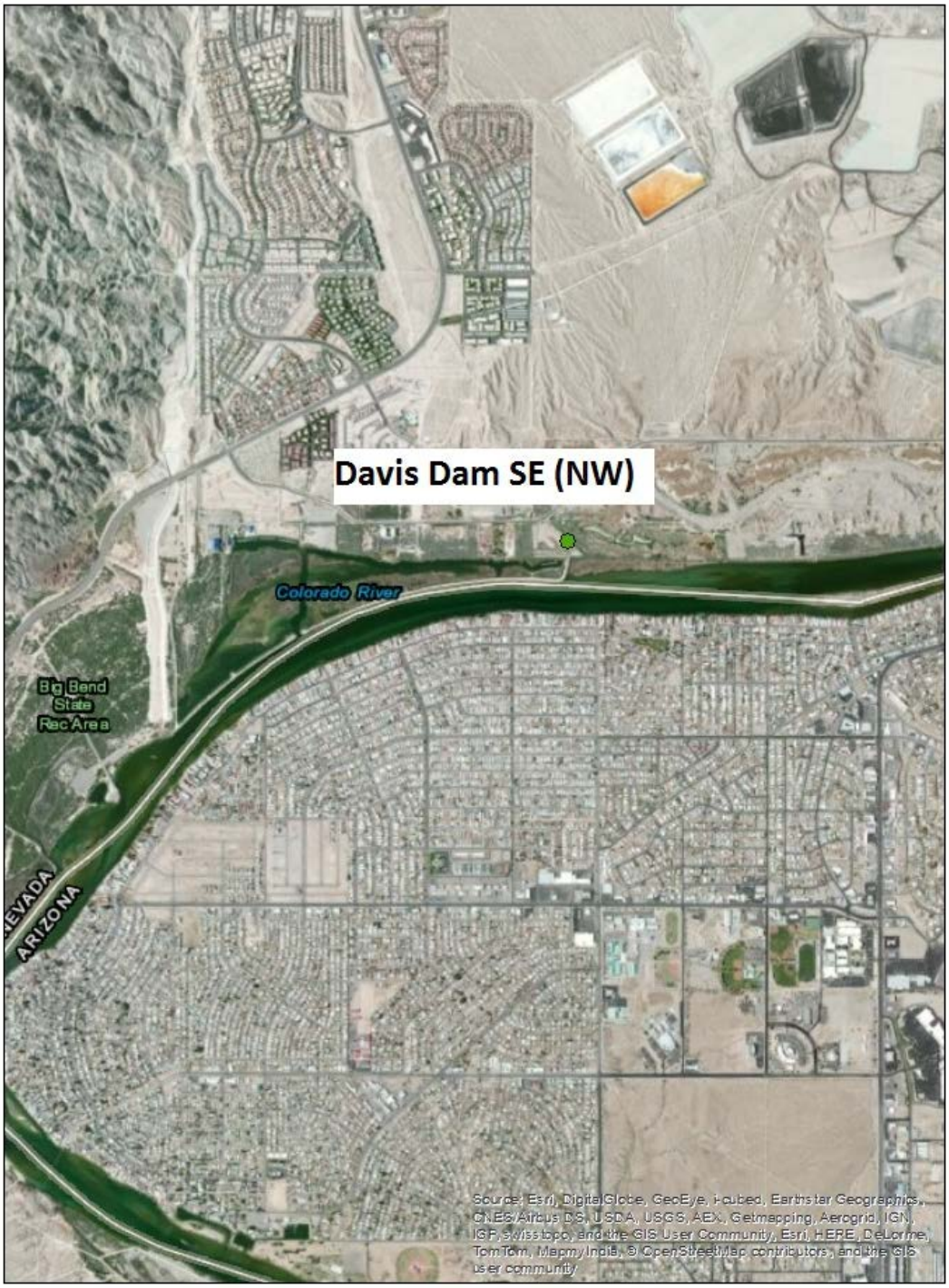

Figure 2-1.—Davis Dam SE (NW) plot location.

Figure 2-2.—Needles (NW) plot location.

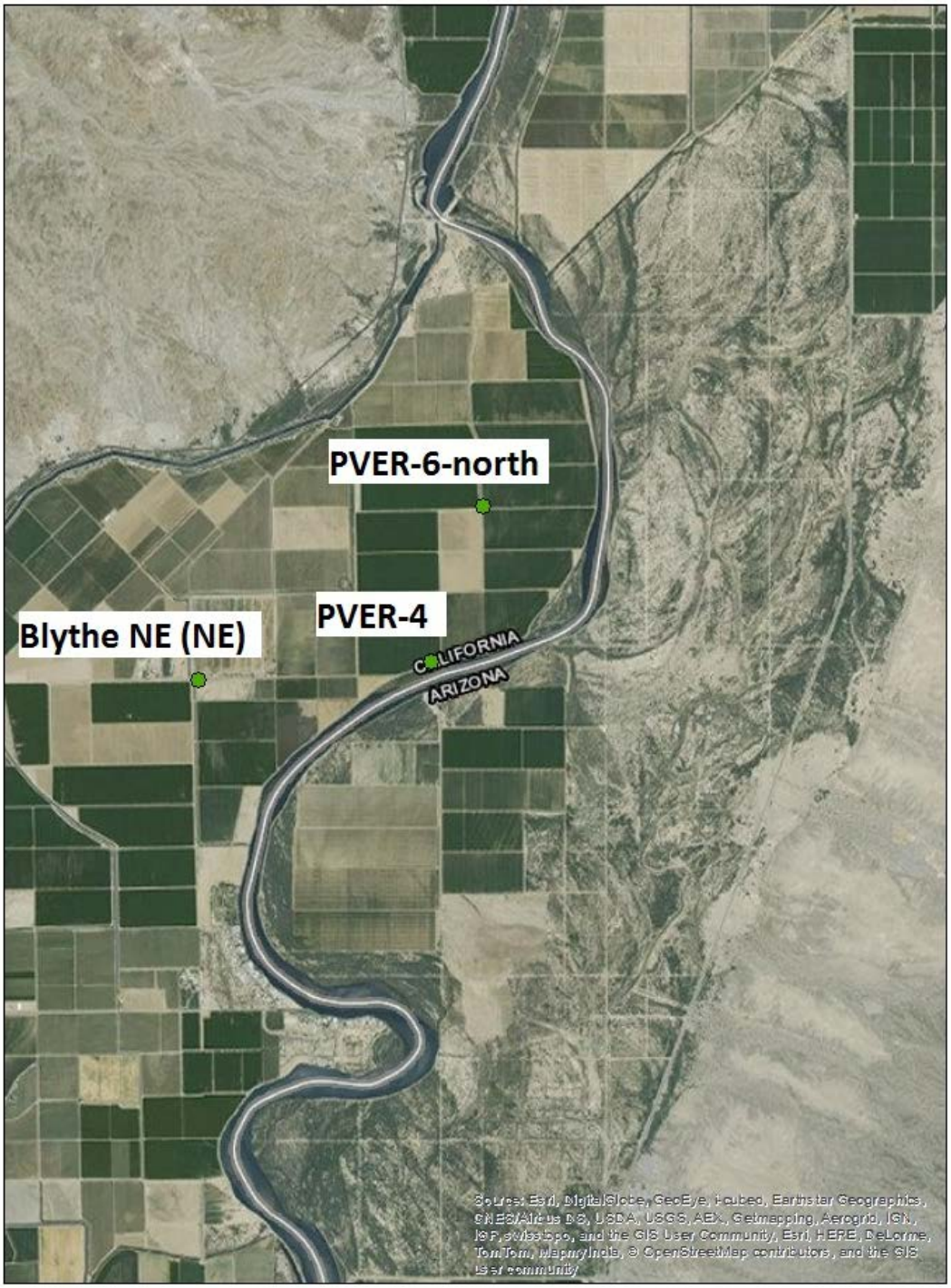

Figure 2-3.—PVER-4, PVER-6-north, and Blythe NE (NE) plot locations.

Figure 2-4.—CVCA-4-west-north plot location.

Figure 2-5.—Cibola and Hart Mine Marsh plot locations.