# Lower Colorado River Multi-Species Conservation Program

*Balancing Resource Use and Conservation*

## Effects of Abiotic Factors on Insect Populations in Riparian Restoration Sites, 2008 Annual Report

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

April 2010

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**Lower Colorado River**
**Multi-Species Conservation Program**
**Bureau of Reclamation**
**Lower Colorado Region**
**Boulder City, Nevada**
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**April 2010**

# Abstract

I examined the effects of nitrogen fertilizer on arthropod abundances and biomasses on *Salix exigua* and *Populus fremontii* at the Palo Verde Ecological Reserve in 2008. Urea fertilizer (1.1 kg, 46% N) was applied to 16 shrubs or trees of each species. Two branches from different unfertilized and fertilized plants were cut once per month during May-August and sampled for arthropods, branch water content, and leaf nitrogen content. Fertilizing plants of both species increased concentrations of nitrogen in leaves and water in branches. Abundances and biomasses of arthropods combined were not affected by fertilization. Within Arthropoda, Araneae (spiders) and Heteroptera (bugs) abundance and biomass also were not affected. Plant fertilization did increase abundances and biomasses of Homoptera (e.g., leafhoppers and aphids). Homoptera comprised 11% of total arthropod biomass. Adding nitrogen fertilizer to shrubs and trees planted for wildlife will cause a small, but significant, increase in the arthropod prey base for birds and bats.

# Introduction

Eight species of birds (southwestern willow flycatcher, yellow-billed cuckoo, gilded flicker, Gila woodpecker, vermilion flycatcher, Bell's vireo, Sonoran yellow warbler, summer tanager) and four species of bats (western red bat, western yellow bat, California leaf-nosed bat, pale Townsend's big-eared bat) included in the Lower Colorado River MSCP eat arthropods (spiders and insects). Creating and maintaining habitat for these species will require providing an adequate supply of arthropods for food. This is especially difficult at several MSCP habitat creation sites being developed, because riparian vegetation is being planted in non-riparian farmland (i.e., where water tables are lowered, soil salinities are elevated, and spring flood flows are absent). Growing plants will not by itself guarantee arthropod abundances large enough to feed and support bird and bat populations. Two abiotic factors, plant water content and plant nitrogen content, greatly influence abundances of plant-feeding insects (Bernays and Chapman 1994). Nitrogen is especially limiting, because plants contain approximately 3% nitrogen (% of dry weight) and insects contain approximately 10% nitrogen. Insect survival and fecundity increases with increasing levels of plant water and nitrogen (Scriber, 1984). Levels of plant water and nitrogen can be manipulated, depending on soil conditions, by controlling plant irrigation and fertilization.

The primary objective of this work task is to determine the response of insect abundance to plant water and nitrogen contents in created riparian habitat. We initiated this work during 2006 by developing a method for measuring plant nitrogen at Reclamation's Regional Laboratory in Boulder City. During 2007, I performed a preliminary investigation of the effects of nitrogen fertilizer on arthropod populations at the Mass Transplanting Site, Cibola NWR (see C5 2007 Annual Report). This study was inconclusive, because the amount of fertilizer applied was inadequate to increase leaf-nitrogen concentrations. I repeated the study during 2008 at the Palo Verde Ecological Reserve and applied greater quantities of nitrogen fertilizer. Results of this work are reported here.

# Study Area

The study was conducted at Phase 2 of the Palo Verde Ecological Reserve. Phase 2 is a 21.4-ha farm field that was planted with riparian trees and shrubs during 2007. Contained in the site are 3.5 ha of *P. fremontii* trees planted in two blocks: 6.6 ha of *S. exigua* shrubs planted in seven blocks, and 6.6 ha of *Salix gooddingii* trees planted in seven blocks. Alfalfa was planted throughout the plot to provide an understory. Trees and shrubs are in north-south rows, and the plot was flood irrigated from north to south every 7-10 days during the study. I sampled shrubs and trees at two locations 0.3 km apart from east to west within the site. Each location supported one block of *P. fremontii* trees that were 4-5 m tall and adjacent to a northerly block of *S. exigua* shrubs that were 2-3 m tall. *Salix exigua*, but not *P. fremontii*, were flowering or seeding.

# Methods

I randomly selected two rows of plants 20 m apart at each location. I randomly selected one plant of each species in each row and then flagged every other *P. fremontii* tree and every third *S. exigua* shrub along the row until eight plants of each species were marked. I shredded the understory beneath the canopy of each flagged tree or shrub with a string-trimmer. I randomly-selected one row at each location and raked 1.1 kg of granular urea fertilizer (46% N) into the shredded debris beneath each flagged plant within the row on 7-8 April 2008. I applied fertilizer to plants in the same row to prevent it from spreading to non-fertilized plants by irrigation. This produced eight plants in each treatment (fertilizer applied or not applied) in each species at each location.

Arthropods on plants were collected monthly during May-August 2008. I collected arthropods with a 137-cm long, fine-mesh net. I swept the net over a 1-m long branch and constricted it around the base of the branch. I fumigated the enclosed branch with an aerosol insecticide containing 0.2% tetramethrin and 0.4% permethrin (Hot-Shot Fogger, United Industries, St. Louis, MO) to knock down or kill captured arthropods. I shook the branch to dislodge the arthropods into a snap-cap plastic tube attached to a cut corner of the net. I cut the base of the enclosed branch, removed the branch from the net, and weighed it with a spring scale to estimate arthropod sample-size. I cut one or two side branches, approximating one-tenth of the branch mass, from the branch and weighed them with a spring scale. I stored the side branches in paper bags for later nutrient analyses. I sampled one branch from one randomly-selected tree or shrub in each species in each fertilizer treatment at each location during the same morning. I took samples from different plants on 16 and 27 May, 9 and 10 June, 7 and 8 July, and 11 and 12 August 2008.

I measured concentrations of water and leaf-nitrogen in side branches. The percent water of each side-branch sample was estimated by reweighing it after drying 24 h at 80ºC. I estimated the percent nitrogen of leaves in each side-branch sample by Kjeldahl digestion (Isaac and Johnson 1976). A 25-30 mg sample of dried, ground, and sieved leaves was heated with a block digestor 1 h at 400ºC in 7 ml of concentrated sulfuric acid, containing 4.2% selenous acid, and 3 ml of 30% hydrogen peroxide. Water was added to 50 ml, and the ammonia concentration of the supernatant was measured against standards by colorimetry with a segmented flow analyzer (OI Analytical, College Station, TX). I repeated the procedure on a second sample of dried leaves and averaged percentages of leaf nitrogen (transformed $2\ \arcsin[\%N/100]^{1/2}$) across the two measurements.

Arthropods from each netted branch were transferred into 70% ethanol and classified by taxonomic order in Araneae (spiders), Collembola (springtails), Psocoptera (psocids), Thysanoptera (thrips), Coleoptera (beetles), Lepidoptera (butterflies and moths), or Hymenoptera (wasps and bees) or by suborder Heteroptera (bugs) or Homoptera (e.g., leafhoppers, psyllids, aphids) in Hemiptera or suborder Nematocera (long-horned flies) or Brachycera (short-horned flies) in Diptera. Arthropods in each taxon were counted and weighed (wet mass ± 0.1 mg) with a microbalance (C-30, Cahn Instruments, Cerritos, CA).

Abundances and biomasses (each transformed log[$Y$ + 1] to normalize residuals) of all arthropods and of arthropods in each of the most-abundant taxa (Araneae, Heteroptera, and Homoptera) were compared within plant species with analysis of variance (Neter et al. 1996, calculated with SYSTAT version 10.2, Richmond, CA). I included locations, and interaction between fertilizer treatment and sampling month, in the models to reduce error variances. I controlled for varying arthropod-sample sizes by including masses of netted branches as a covariate in each analysis of variance.

Relationships between plant measurements and quantities of Homoptera, the only taxon that responded to fertilizer treatment, were examined in each plant species. I separately regressed the transformed abundance and biomass of Homoptera on each netted branch against the percentage of branch water and the transformed percentage of leaf nitrogen in each side-branch sample. I included netted-branch mass as a predictor in each regression to control for different arthropod sample-sizes.

# Results

Applying 1.1 kg of nitrogen fertilizer to each shrub or tree significantly increased percentages of leaf nitrogen and branch water in both species. Means are presented in Table 1.

**Table 1.**

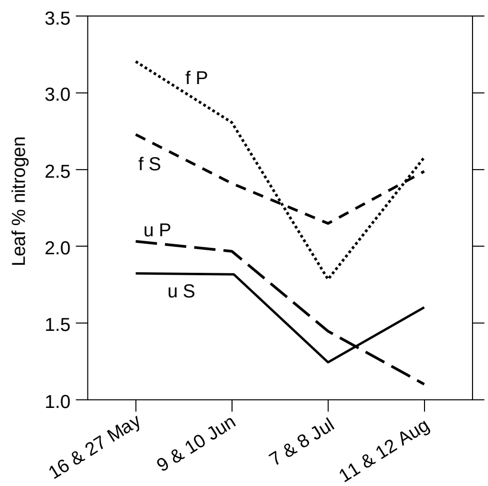
| | *Salix exigua* | | *Populus fremontii* | |
|---|---|---|---|---|
| Estimate | Unfertilized | Fertilized | Unfertilized | Fertilized |
| Branch % water | 64.4 | 66.5 * | 68.1 | 70.1 * |
| Leaf % nitrogen | 1.6 | 2.4 * | 1.6 | 2.6 * |

* Significant increase on fertilized plants, $P < 0.05$.

Nitrogen concentrations in *S. exigua* leaves were 19% higher (% change in mean of transformed % N) on fertilized shrubs compared with unfertilized shrubs. *Populus fremontii* leaves contained nitrogen concentrations that were 21% higher on fertilized trees compared with unfertilized trees. Water contents in *S. exigua* branches were 3.2% higher on fertilized shrubs compared with unfertilized shrubs. *Populus fremontii* branches contained water contents that were 2.9% higher on fertilized trees compared with unfertilized trees.

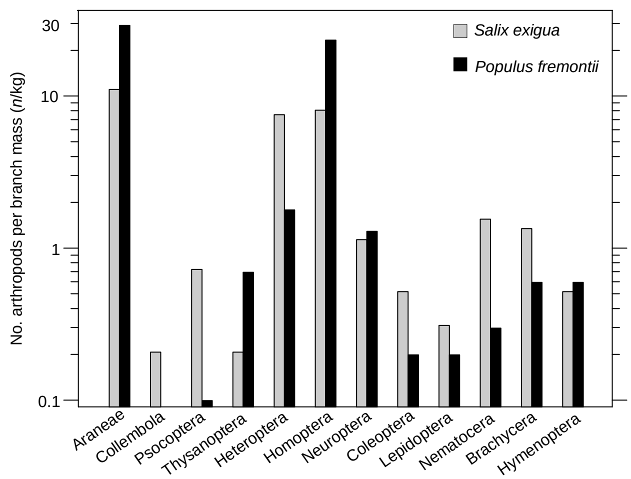
Concentrations of nitrogen in leaves tended to decrease across sampling months on unfertilized and fertilized *P. fremontii* trees, whereas concentrations in *S. exigua* leaves were relatively constant. For Figure 1, u = unfertilized, f = fertilized, S = *S. exigua*, and P = *P. fremontii*.

**Figure 1.**



I collected 322 arthropods on unfertilized and fertilized *S. exigua* shrubs and 587 arthropods on unfertilized and fertilized *P. fremontii* trees across the four dates (Figure 2).

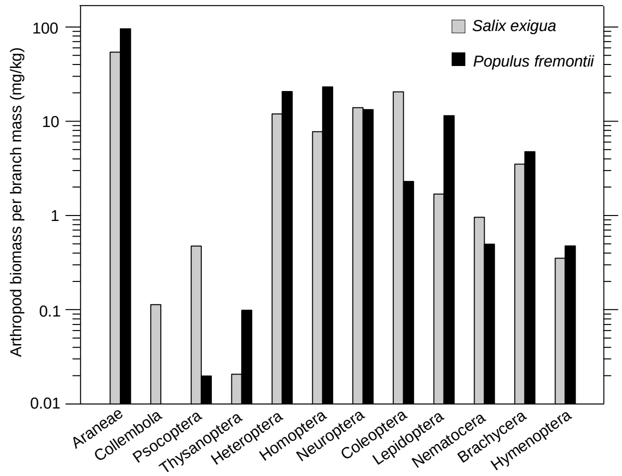
**Figure 2.**




Spiders (Araneae) were the most abundant taxon on both plant species, comprising 44% of total arthropod abundance. Most spiders collected were immatures. Most adult spiders on *S. exigua* and *P. fremontii* were two-clawed hunting spiders (Clubionidae) followed by jumping spiders (Salticidae). The most abundant insects on *S. exigua* and *P. fremontii* were Homoptera followed by Heteroptera and Neuroptera. These three taxa comprised 34%, 11%, and 3% of arthropod abundance, respectively. Most Homoptera on *S. exigua* and *P. fremontii* were leafhoppers (Cicadellidae), and aphids (Aphididae) also were abundant on *S. exigua*. Leafhoppers and aphids are herbivores. The most-abundant family in Heteroptera collected from both plant species was leaf bugs (Miridae), typically herbivorous, followed by minute pirate bugs (Anthocoridae), typically predaceous. Neuroptera were primarily green lacewings (Chrysopidae) collected as adults and predaceous larvae. After Neuroptera, the most abundant insects were Brachycera followed by Nematocera, Hymenoptera (macro- and micro-wasps), Thysanoptera, Coleoptera, Lepidoptera (moths), Psocoptera, and Collembola.

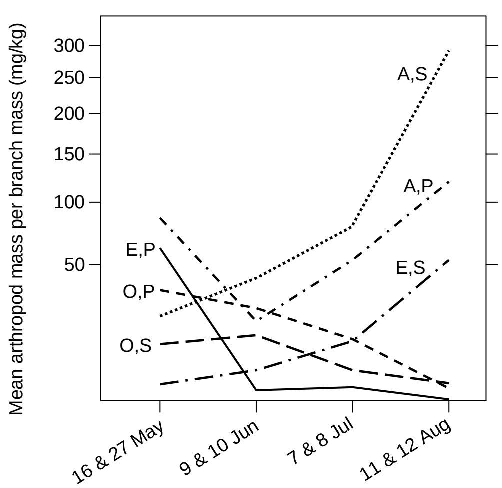
Wet masses of arthropods totaled 1.119 g on *S. exigua* and 1.741 g on *P. fremontii* and generally corresponded with abundances. Most arthropod biomass (51%) on both plant species was spiders. The average body mass of spiders was 4 mg. Homoptera and Heteroptera each comprised 11% of arthropod mass, and Neuroptera accounted for 10%. The average body mass of Heteroptera (3 mg) was greater than that of Homoptera (1 mg). Biomasses of collected taxa are presented in Figure 3.

**Figure 3.**




Biomass of spiders was greater late in the season, whereas biomass of Homoptera was greater early in the season (Figure 4).

**Figure 4.**



Fertilizer treatment did not significantly influence abundances or biomasses of arthropods combined, spiders, or bugs on *S. exigua* or *P. fremontii*. In contrast, Homoptera increased in abundance and biomass on fertilized plants in both species. Means are presented in Table 2.

**Table 2.**

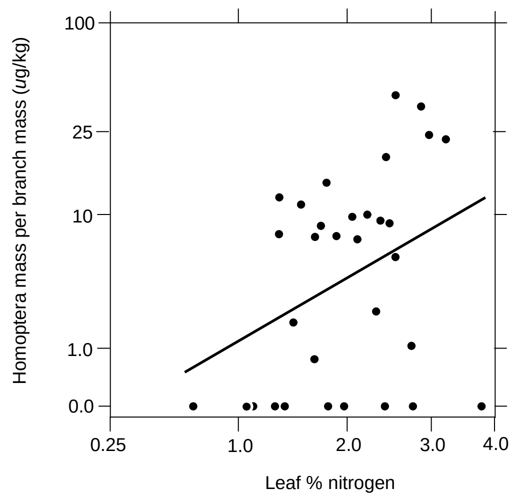
| | *Salix exigua* | | *Populus fremontii* | |
|---|---|---|---|---|
| Estimate[a] | Unfertilized | Fertilized | Unfertilized | Fertilized |
| Arthropoda | | | | |
| Abundance (*n*/kg)[c] | 29.2 | 29.5 | 32.9 | 50.0 |
| Wet mass (mg/kg)[c] | 62.9 | 72.7 | 65.3 | 108.5 |
| Araneae | | | | |
| Abundance (*n*/kg)[c] | 14.0 | 15.8 | 13.3 | 8.8 |
| Wet mass (mg/kg)[c] | 37.2 | 35.9 | 35.9 | 18.5 |
| Heteroptera | | | | |
| Abundance (*n*/kg)[c] | 5.5 | 4.9 | 1.0 | 1.5 |
| Wet mass (mg/kg)[c] | 7.8 | 4.6 | 0.8 | 3.2 |
| Homoptera | | | | |
| Abundance (*n*/kg)[c] | 2.8 | 8.0 * | 4.7 | 13.9 * |
| Wet mass (mg/kg)[c] | 6.7 | 19.9 * | 10.6 | 34.8 * |

* Significant increase on fertilized plants, $P < 0.05$.

Percent changes in abundance and biomass were similar between plant species. Homoptera on *S. exigua* increased 52% in abundance and 45% in biomass (% change in mean of transformed *n* or mg). Homoptera on *P. fremontii* increased 52% in abundance and 46% in biomass.

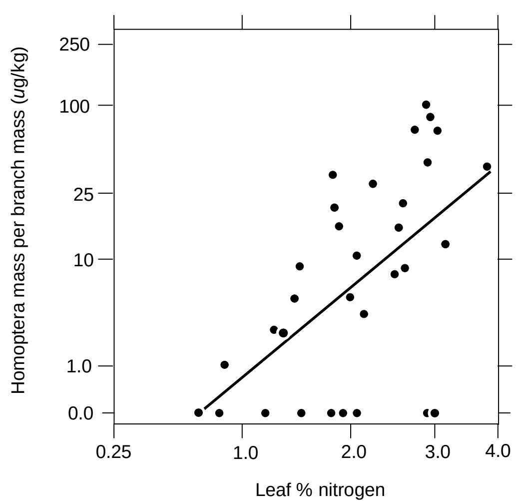
Biomasses of Homoptera increased ($P = 0.047$, $R^2 = 0.15$) on *S. exigua* branches as concentrations of nitrogen in leaves increased (Figure 5).

**Figure 5.**




Biomasses of Homoptera were more strongly related to concentrations of nitrogen in leaves ($P = 0.001$, $R^2 = 0.55$) on *P. fremontii* (Figure 6).

**Figure 6.**



# Discussion

Fertilizing *S. exigua* shrubs and *P. fremontii* trees with nitrogen fertilizer increased concentrations of nitrogen in leaves and, unexpectedly, concentrations of water in branches. Fertilized plants likely produced more roots and were better able to absorb soil water. Nitrogen concentrations in leaves also trended downward during the season in unfertilized and fertilized plants. Nitrogen concentrations in leaves were highest in May, suggesting that leaves lose nitrogen as they senesce. One study on aspen (*Populus tremuloides*) in New York suggested that increased nitrogen provided by fertilizer increased leaf growth more than leaf-N content. The proportion of applied fertilizer that was absorbed into each plant in the present study is unclear. A significant amount of urea fertilizer likely was transported laterally from fertilized plants, or leached downward below the root zone, by irrigation water. My method of applying fertilizer was relatively crude and can be improved for applications over a large area.

Fertilizing trees with urea also increased abundances and biomasses of Homoptera, including leafhoppers and aphids. All Homoptera are phytophagous. Leafhoppers and aphids are especially sensitive to leaf nitrogen-contents, because they generally feed on phloem. Their food source therefore contains high concentrations of water and sugar, and low concentrations of nitrogen (present in phloem as amino acids). Homoptera also were most common in May when nitrogen contents in plants were highest. Heteroptera contains species that are either phytophagous, such as Miridae, or predaceous, such as Anthocoridae. The varied diets in bugs likely precluded detecting an influence of plant nitrogen on their abundances or biomasses.

Spiders are only predaceous, and their populations did not respond to fertilizer treatment. Increased biomass of herbivorous insects, such as Homoptera, expectedly would have extended up the food chain and caused greater biomass of spiders. The absence of this effect suggests spiders are not exclusively reliant on insects developing on the plant, but may also feed on insects migrating into the plot of planted trees and shrubs. Biomasses of spiders exceeding those of insects also were observed in the Mass Planting Site during 2007 (see C5 2007 Annual Report).

Insectivorous birds can have varied diets. Our work on the southwestern will flycatcher (Wiesenborn and Heydon 2007) found birds eating a diverse array of spiders and insects including 57 taxa in 32 families and eight orders. Flycatchers ate Miridae and Cicadellidae, and cicadellids were the most abundant prey item found in flycatcher fecal samples. In the present study, leafhoppers increased in abundance and biomass on fertilized willows and cottonwoods. Their biomasses also were positively related to concentrations of leaf nitrogen. Supplying willows and cottonwoods with adequate nitrogen is important in maximizing abundances and biomasses of arthropod prey for birds and bats.

# Recommendations

1. Concentrations of nitrogen in leaves should be monitored to ensure maximum arthropod production for birds and bats. I am not able to provide an accurate target value for leaf percent-nitrogen from these results. Nitrogen concentrations generally exceeded 2.5% in fertilized *S. exigua* and *P. fremontii*. Irrigated, fertilized aspen in New York also supported leaves containing 2.5% nitrogen (Funk et al. 2007). Nitrogen concentrations > 2.5% therefore appear to be a reasonable, initial estimate for growing adequately fertilized plants.

2. I have not been able to examine the effects of irrigation regime on arthropod populations in this study, or in 2007, due to the frequent irrigations (every 7-10 days in 2008) provided to plants. Plant water content can be critical to arthropod development. Effects of irrigation on arthropods should be investigated if irrigation frequencies are reduced.

3. Spiders and insects should be monitored within restoration sites to ensure adequate prey bases are being provided to birds and bats. Spiders and insects likely will need to be monitored differently.

4. Due to the greater biomass of spiders compared with insects, the contribution of immigrant insects as food for wildlife should be quantified. Plot location, such as next to Topock Marsh where aquatic insects immigrate into riparian habitat (Wiesenborn and Heydon 2007, 2008) may be important to prey base establishment.

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