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Final Report for Effectiveness Monitoring of Springfed Wetlands and Riparian Restoration Treatments: Progressive Management of Invasive Tamarisk in the Southern Nevada Region

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EXECUTIVE SUMMARY

Effective management of non-native plant invasions that threaten native vegetation and associated wildlife species depends on control treatments that provide sustained weed reduction and facilitate their replacement with self-replicating native and/or beneficial non-native plants. This research project involved an Effectiveness Monitoring assessment of riparian locations in which actions to control invasive weeds, particularly tamarisk but including other noxious taxa, have been applied in Clark County and adjacent regions. Research was to characterize and evaluate control methods (chemical and mechanical treatments, fire, biological) to determine management approaches that yielded, or will yield, desired results (recovery of native vegetation and wildlife habitat, improved substrate condition, reduced wildfire risk) while resisting re-invasion by secondary noxious weeds.

The effects of simulated biological control of tamarisk were to be addressed as part of the original project to understand the potential impacts and responses when and if biocontrol agents were introduced into Clark County. However, the unanticipated introduction of the tamarisk leaf beetle, *Diorhabda carinulata*, into the project area during the course of this research provided the opportunity and need to evaluate biological control as a weed management method and its potential to facilitate ecosystem recovery from exotic species invasion, and subsequent improvement of habitat for Covered and other riparian dependent species.

Goal and Objectives of the Project

Thus, the Goal of this multi-agency research program was to promote adaptive weed management that provides optimal, cost-effective weed control with a minimum of collateral impacts to associated beneficial species, and integrates ecosystem functions into the habitat recovery process. The overall Objective was to undertake an extensive evaluation of regional infestation sites, those where tamarisk has been treated and those where treatments were anticipated, to understand the underlying interrelationships among species and environmental processes, including physical disturbance, fire (natural and prescribed), and hydrologic conditions.

The specific Objectives of the research project are as follows:

1. Conduct a multivariate study evaluating relationships among current tamarisk dominance, dominance of other non-native and native plant species, wildlife habitat structure and wildlife associations, tamarisk control treatments, fire, and other environmental variables to assess effectiveness of weed control and riparian vegetation restoration treatments.

2. Compare vegetation structure and composition, and tamarisk flammability in relation to foliage condition, in response to experimental herbicide treatments to simulate anticipated defoliation effects of tamarisk biocontrol.

3. Compare mortality of tamarisk following fire with and without previous herbicide treatments.

4. Evaluate herbicide and cultural treatments for the control of Russian knapweed, a recent invasive species that responds positively to disturbance and may function as a surrogate for treatment of other secondary weed invasions.

5. Monitor dispersal and establishment of the tamarisk leaf beetle, document life cycles, genetic changes, and factors that may limit its establishment (predation, premature winter diapause, extreme temperatures); assess effects of this biological control agent on tamarisk condition, defoliation-re-growth dynamics and responses by associated vegetation and wildlife to tamarisk defoliation; evaluate fire risk dynamics related to defoliation.

Vegetation Responses to Treatments (Objective 1) This multi-disciplinary project encompassed several research elements, the primary objective targeting vegetation responses to prior tamarisk control treatments carried out by NPS and BLM during the prior decade in floodplain systems, particularly the Virgin River, and in regional upland spring wetlands. Primary findings were that the tamarisk control treatments in floodplains have been effective in significantly and strongly reducing tamarisk stem densities and vegetative cover (from 25% to 1.3% total woody plant cover) compared with untreated control sites, and opening the canopy with much greater areal cover of open space (45% vs. 5% as expected less than 5 years following mechanical treatments. These reductions in tamarisk cover are associated with significant increases in diversity (53 vs. 32 species) and vegetative cover of native shrub and tree species, although herbaceous species did not show significant differences in density nor cover. However, the physical disturbance associated with these treatments also promoted invasion and/or expansion of weedy plant species such as Russian thistle (tumbleweed; *Salsola tragus*) that can interfere with native plant recruitment, and provide poor habitat for wildlife. Some non-native species, particularly grasses (*Bromus* spp. and *Schismus barbatus*), were more common in association with tamarisk in untreated areas so treatments can reduce multiple invasive species. In upland spring sites, time since treatment has a major role in promoting tamarisk reduction and native woody plant increases. Sites initially treated >6 years prior to surveys had lower tamarisk density and cover than more recently treated sites, primarily because NPS policy is to return to sites for follow-up treatments. Native woody plants showed minor enhancement over time, while herbaceous species were significantly more abundant in relation to time since initial treatment.

These results indicate that traditional methods of tamarisk removal have mixed effects. They have been effective in meeting some objectives, particularly increasing diversity and abundance of native plants, as well as reducing the likelihood of wildfire (sparser distribution of plant biomass, or 'fuels' in treated area) [Fuels management was the primary driver of weed control/restoration treatments in non-NPS locations.] However, a general conclusion is that conventional treatments have the undesirable effect of increasing the abundance of other noxious, non-native species that can interfere with native vegetation recovery. Ecosystems managed for invasive plant control will need repeated attention, and probably active restoration of important taxa, to eventually reach to goal of sustainably increasing native species abundances and diversity in Clark County wetlands. Restoration efforts by BLM, and by our research team, yielded positive short-term results on the Virgin River in terms of plant survival, except where livestock grazing inhibited establishment. However, flooding in December 2010 largely destroyed restoration treatments before plants could mature and illustrated how future restoration actions must be based on a comprehensive evaluation of where site conditions and flood-path probability are anticipated to facilitate sustained establishment of native vegetation.

The role of prescribed- and wild-fire in potentially reducing tamarisk cover and density was part of this Objective, but is more cohesively addressed in subsequent sections (Objectives 2 and 3).

Tamarisk Treatments and Wildlife Responses (Objective 1) Avian studies indicated that, in general, bird diversity and abundances were substantially lower in locations where mechanical-chemical tamarisk treatments have been conducted than in untreated areas where tamarisk dominance remained high. In the absence of active restoration measures to provide large-statured native riparian vegetation, treated sites had lower structural habitat available to wildlife, thus a simplifying of the vegetation assemblage and consequent simplification in the avian assemblages. At least in the short term since treatments were conducted (up to 4 years prior to avian surveys), vegetation structure had not substantially improved. This had negative impacts on many taxa, including Covered and regionally sensitive species. Some species such as loggerhead shrike that are generally associated with open habitat are, however, positively affected by such conditions. Nest-parasitizing cowbirds were also more prevalent in untreated sites that had higher tamarisk dominance, which has management implications for sensitive native birds. These results suggest that tamarisk removal by traditional methods alone, in the absence of appropriate conditions for re-establishment of woody native plants, may not be a desirable approach to riparian management if the objective is to improve habitat for native wildlife. To maintain avian community persistence and species diversity in a post-tamarisk-removal environment, it is advisable to include active vegetation restoration as a management prescription.

A subsequent study evaluated the relationship between vegetation types, specifically tamarisk monoculture vs. mixed stands composed of tamarisk with a significant native woody plant component, with respect to value as avian habitat. This is relevant to expectations of avian response to tamarisk reduction by biological control and subsequent recovery of native elements, as opposed to increasing dominance of riparian systems by tamarisk. Birds are less diverse and abundant in tamarisk monocultural habitat when compared with habitat that is still dominated by tamarisk but with a substantive native component. Most species showed a clear preference for native habitats. Nonetheless, a number of bird species do use tamarisk regularly during the breeding season, and in general tamarisk is not lower quality nesting habitat than native habitat for many. Nest failure and cowbird parasitism rates were lower as tamarisk prevalence increased, possibly because native vegetation may also be more attractive to predators and nest parasites. Tamarisk removal programs should take into account that its large-scale reduction can have some deleterious effects on bird communities, at least in the short term, and should consider active restoration in areas where native recovery may be slow or impractical.

Other wildlife studies conducted in collaboration with our project found similar relationships with tamarisk-dominated vs. mixed vegetation for herptiles and small mammals but will not be reported here. However, bat studies were partially supported by this project, and a preliminary vocalization detection analysis of several vegetation types indicated that at least 17 species were present in the Virgin River system. Bats favored native-dominated sites in both species diversity and abundance. Bats would be important to include as a key wildlife response variable for projects in the Clark County region, and some are already recognized as species of conservation concern.

Tamarisk and Wildfire (Objectives 2 & 3) A major concern regarding tamarisk invasion of Clark County riparian ecosystems is that it is considered a serious wildfire hazard. A series of integrated field and laboratory studies illustrate the relationships between tamarisk infestation, fire impacts to native vegetation, and use of prescription fire to manage tamarisk, including how these relationships may be altered by the introduction of biological weed control. First, findings illustrate that tamarisk is more flammable than native species, regardless of foliar condition. Then, in an extensive survey of tamarisk-fueled wildfires across the southwestern deserts (also addressing the role of fire from Objective 1), native plant tissues (foliage/stems/trunks) are more fully consumed by fire, and post-fire native plant recovery declines, as the density of tamarisk increased. This indicates a positive feedback system where tamarisk promotes its own post-fire recovery and expansion, and further displaces native vegetation, illustrating the management importance of reducing the relative abundance of tamarisk in regional ecosystems.

When tamarisk foliage is desiccated (by herbivory or herbicide simulation of herbivory), it exhibits more extreme fire behavior (greater flame lengths and rate of spread) and burns with only slightly greater intensity than live, green foliage under fire weather conditions. Thus, tamarisk is clearly an important fire threat in riparian systems, including when it is green and actively growing. Prolonged herbivory by the tamarisk leaf beetle, *Diorhabda carinulata*, the species present in Clark County, causes incremental die-back and reduced foliar volume, and can lead to mortality after 3 or more years owing to diminished metabolic carbohydrate reserves. The combined effects of herbivory and fire lead to enhanced mortality of invasive tamarisk so the presence of this biocontrol agent in Clark County offers beneficial potential for reducing long term fire risk even if flammability may be slightly increased during early establishment.

Effectiveness of Treatments for Secondary Weeds (Objective 4) To anticipate the secondary invasion by other weedy species following tamarisk control efforts, Russian knapweed (*Acroptilon repens*) provides a proxy for disturbance-following forbs, and trials using a variety of rangeland herbicides were conducted to determine which gave most effective reductions in density and cover. Replicated trials were conducted during winter dormancy at Ash Meadows National Wildlife Refuge using a variety of rangeland herbicides on Russian knapweed infestations, the active compounds tested including imazapyr , aminopyralid at three concentrations, imazapic, and chlorsulfuron. Preliminary results the following summer indicated that aminopyralid provided greatest efficacy. Intermediate and high doses were more effective than the low dose treatment (all within label-recommended range). Plots were eliminated prematurely by refuge management, prior to intended second year evaluation, but year 1 results suggest a recommendation for medium dose (5 ounces/acre rate) treatment of this, and likely other secondary invasive plants.

Tamarisk Biological Control (Objective 5) The tamarisk leaf beetle, *Diorhabda carinulata*, was released in 2006 into the Virgin River watershed at St. George, UT and during this project proceeded to disperse unassisted in multiple directions, including along the Virgin River in Arizona and Nevada, as well as southward (via Paria Canyon) to the Colorado River where it is now present within the Grand Canyon. The rate and dynamics of its expansion have been documented each year, including recent establishment in 2011 to within 5 kilometers of the Lake Mead 'full pool' in both the Virgin and Muddy River systems. The research team and collaborators recruited to provide broader expertise have established a multi-disciplinary ecosystem monitoring program to track responses of vegetation, ecosystem parameters (evapotranspiration/plant water use and groundwater level, soil salinity, micro-climates), and wildlife over time.

The Project Amendment to evaluate status and responses to tamarisk biocontrol has resulted in an initial baseline dataset documenting *D. carinulata* population size, distribution, life cycles and initial impact to tamarisk vegetation, primarily simple defoliation to-date. Based on concurrent studies in other regions, broad mortality is unlikely to occur for several years but limited mortality has already been observed at some locations, apparently by exhausting metabolic reserves in late season so plants have no capacity to compensate for defoliation and recover via late season re-growth. The beetles typically produce 2 generations per season in this climate zone while a partial third generation was documented prior to induction of over-winter diapause. Laboratory studies suggest that high temperatures achieved in the Mojave Desert could result in sub-optimal growth and development performance, but are unlikely to limit its potential range in the southern Nevada and lower Colorado River region. Laboratory and field studies show that *D. carinulata* has undergone natural selection in the southern Nevada region to synchronize developmental changes (over-winter diapause) with this southern latitude and host-plant growing conditions.

The monitoring data will form the basis for proposed continued documentation of the role of biocontrol and tamarisk suppression in promoting recovery of native vegetation, ecosystem processes and wildlife species, as outlined in appendices related to future responses to this new management treatment.

INTRODUCTION

Description of the Project

Tamarisk (also known as saltcedar; *Tamarix* spp.) and other invasive weeds have been the targeted for control in southern Nevada through several MSHCP Conservation Actions, with numerous control programs, particularly against tamarisk, undertaken in the region. Many of these have a primary focus of managing wildfire risk by reducing fuels from invasive plants such as tamarisk and cheatgrass, while recovery of native vegetation to provide habitat for Covered and other wildlife species is also an important goal. In this report the common name 'tamarisk' refers generally to all species and hybrids belonging to the genus *Tamarix*, and where specific taxonomic identity is necessary we will use the scientific binomial name.

This project involves an extensive survey and effectiveness monitoring program of sites where tamarisk control measures have been applied in Clark County and adjacent regions to identify methods that yielded desired results (recovery of native vegetation and wildlife habitat, improved soil condition, reduced wildfire risk) vs. methods that were ineffective in meeting goals. The project evaluated the alternative hypotheses that 1) reduction in tamarisk density via mechanical and chemical control methods facilitates replacement of invasive species by native riparian vegetation, or 2) some tamarisk control methods have the unintended consequence of promoting invasion and replacement by other noxious exotic weeds. Untreated sites, and tamarisk sites that have experienced wildfire, were surveyed to document relationships between environmental variables and status of tamarisk and of associated native and exotic plants. Best management practices for control of tamarisk and secondary invasive plants (e.g. Russian knapweed) were tested where feasible, including evaluation of prescribed fire to enhance target mortality, reduce fuel loads and wildfire risk, and promote riparian restoration. Native plant restoration trials were conducted to evaluate effective methods for propagation, and to evaluate the impact of grazing on viability.

Biological control of tamarisk was initially simulated to evaluate its potential for weed reduction and wildlife habitat restoration. The introduction of a specialist biological control agent, the tamarisk leaf beetle (*Diorhabda carinulata*), into the Virgin River watershed and subsequently into Clark County during the course of the project created the conditions for monitoring its colonization and initial impacts. In addition, this allowed us to develop a baseline dataset for documenting the biodiversity and ecosystem responses, both negative and positive, to tamarisk biocontrol as it proceeds in this region. Several species of *Diorhabda* are released or under controlled testing in North America, but only *D. carinulata* is present within the Colorado River Basin so all references (including use of the term 'beetle') are to that species except where broader discussion makes reference to other species of *Diorhabda* or more broadly to the whole genus, as will be noted.

Background and Need for the Project

Riparian areas are spatially rare, critically important ecosystems in the deserts of North America, sustaining far great diversity and abundance of wildlife species than any other major habitat type (Skagen et al. 1998, Sanders and Edge 1998) and including a disproportionately large segment of our threatened and endangered species (Brookshire et al. 1996). Many of the rare and declining native plant and animal species Covered in the Clark County Multiple Species Habitat Conservation Plan for southern Nevada depend on these habitats for their continued existence. Although riparian ecosystems in southern Nevada and the rest of the arid West have been extensively degraded by anthropogenic factors, including land development, water manipulation and exotic species invasions (Allan and Flecker 1993, Moyle 1995, Neary et al. 2000), moderately degraded systems do retain many habitat values as well as buffering anthropogenic impacts from contamination and erosion (Sabo et al. 2005, Naiman et al. 1993), and restoration of natural riparian elements in such systems has been a high priority goal for resource managers (Szaro and Rinne 1988).

Riparian ecosystems are susceptible to colonization by non-native plants, particularly landscapes with periodic exposure to flooding and other disturbances that open substrate to invasion (D'Antonio et al. 1995, Hood and Naiman 2000), as regularly occur in flashy desert systems. In southern Nevada and surrounding desert regions most riparian areas have become heavily infested by taxa such as tamarisk, perennial pepperweed/tall whitetop (*Lepidium latifolium*), Russian knapweed (*Acroptilon repens*), giant reed (*Arundo donax*), camel-thorn (*Alhagi maurorum*), Russian olive (*Eleagnus angustifolia*) and many others (Dudley and Collins 1995, Dudley 2007). In fact, non-native species invasions are considered second only to habitat degradation as a cause for native species endangerment and extinctions (Wilcove et al. 1998). Noxious invaders not only threaten many listed and sensitive species covered by the CCMSHCP, but they also have serious socio-economic consequences, interfering with recreation, grazing, and other land-uses, increasing the frequency and severity of wildfire, and reducing the availability of groundwater (Mack et al. 2000, Congressional Budget Office 1993).

Of particular concern in this region is tamarisk, also known as saltcedar, which is commonly reported to have many negative impacts (reviewed in Shafroth et al. 2005, Dudley et al. 2000) such as streamflow depletion owing to its high water demand and evapotranspiration (Smith and Devitt 1996, Sala et al. 1996, Cleverly et al. 2002, Pattison et al. 2010), stream channel narrowing and increased potential for flood damage (Graf 1998), increased soil salinization, displacement of native vegetation and increased frequency and magnitude of wildfire (Busch and Smith 1995) and provision of inferior wildlife habitat (Lovich and DeGouvenain 1998, Hunter et al. 1988, Shafroth et al. 2005). Economic costs of tamarisk in the western U.S. have been estimated as $127-291 million per year (Zavaleta 2000). For these reasons it has been specifically targeted for control throughout the Southwest (e.g. Kunzmann et al. 1989, Barrows 1998, Anderson and Ohmart 1984, Shafroth et al. 2005).

Invasive Plant Control and Riparian Restoration in Clark County

The above concerns have also been the basis for numerous invasive plant control projects in Clark County, largely implemented by the Exotic Plan Management Team and coordinated through the interagency Southern Nevada Restoration Team (led by C. Deuser, NPS), and scaled up with efforts to control the larger populations along the major riverine systems, particularly the Virgin River where significant control work by federal resource managers was undertaken before and after the 2005 El Nino flood events (Tim Rash and Nora Caplette, BLM, personal communication). Stands of tamarisk are sources of abundant seed to colonize scoured channels, as well as upland springs and tributary systems unless they are actively managed. In addition, because of the great areal extent and dominance of many tamarisk stands in riverine systems, these stands result in some of the most significant impacts of invasive plants on ecosystem processes (e.g. water loss through evapotranspiration, major wildfires) and wildlife habitat use (Shafroth et al. 2005, Hultine et al. 2010, Dudley and Bean 2011). Accordingly, millions of dollars have been, and will be spent on invasive plant control projects in riparian ecosystems in southern Nevada, with emphasis on the Virgin River ecosystem because of its ecological importance in the region, and because the substantial hydrological integrity that it retains (the Virgin River and tributaries are not extensively regulated and still experience periodic, natural flood events) promotes greater biodiversity potential than do most Mojave Desert riparian systems. Costs of mechanical control range from $1,500 to $1,700 ha depending on the technique and difficulty of extraction or removal off-site (Shafroth et al. 2005), so cost-effectiveness depends on assurance that the treatments themselves are effective in facilitating restoration objectives.

Unfortunately, moderate to intense habitat disturbances caused by invasive plant control techniques (e.g. root–plowing tamarisk or broad application of herbicides) may have significant negative effects on native species as well. Many invasive species are at a competitive advantage when soils have been mechanically disturbed or native plants have been stressed by herbicides (D’Antonio et al. 1995). These disturbances caused by control efforts may therefore facilitate secondary invasions by other species (e.g. Russian thistle or cheatgrass invasion after tamarisk control). As a result, active restoration of native vegetation may be an important next step to ecosystem recovery. Rapid re-establishment of native plants can preclude re-invasion by treated invasive species, or secondary invasions by new invasive species. Although restoration of native species may not be warranted in smaller scale invasive plant control projects where control methods are less intrusive and native plant propagules can readily recolonize from adjacent undisturbed areas, the removal of large stands of tamarisk that typically exist in riverine systems may significantly benefit from subsequent restoration of natives.

Alternatively, once a non-native plant such as tamarisk becomes a dominant element in an landscape, it potentially provides adequate, albeit possibly inferior habitat for native species, including federally listed taxa. This has been the case with the Southwestern willow flycatcher (*Empidonax traillii extimus*) in southern Nevada and the Southwest, including the Virgin and Muddy River watersheds where the bird is known to nest in tamarisk (McLeod and Koronkiewicz 2010), leading to uncertainty regarding how to manage abundant invasive species and regulatory conflicts when confronted with competing management goals. This is further complicated when anticipated ecosystem responses to weed management are likely to be fundamentally different in the short term (e.g. temporary loss of nesting habitat) vs. long-term (e.g. enhanced native vegetation, reduced water loss, lowered risk of flood and fire risks)(Dudley and DeLoach 2005, Paxton et al. 2011).

The conflict regarding competing tamarisk management concerns is particularly relevant in Clark County watersheds because of the presence of the tamarisk leaf beetle. Introduced in 2006 into the Virgin River watershed at St. George, Utah for the biological control of tamarisk, this specialist herbivore from Asia has expanded and is defoliating tamarisk through much of the watershed as well as the mainstem Colorado River (Dudley and Bean 2011). The heightened concerns regarding potential loss of nesting habitat for the SW willow flycatcher has led to a lawsuit against the USDA Animal and Plant Health Inspection Service (APHIS) and US Fish & Wildlife Service demanding a halt to the program. That lawsuit, which has terminated or delayed major elements of the tamarisk biological control development program, will be resolved only through documentation of the process and consequence of biocontrol where the leaf beetle currently is found. The Virgin River is the only location where the endangered flycatcher and the tamarisk leaf beetle presently co-occur (Paxton et al. 2011), which has brought national attention to the situation and amplifies the relevance of Clark County regarding future tamarisk management; in fact, the regulatory conflict has delayed biocontrol programs for other invasive species so this project, and others borne from it, have important ramifications for invasive species management and biodiversity conservation nationwide.

Based on objectives of the MSHCP to reduce the threats that invasive plants pose to the species covered under the Plan (poor habitat, hazardous fuels, decreased groundwater levels, and other forms of habitat degradation), it is valuable to document whether and how these control measures lead to increases in the abundance and structural dominance of native perennial vegetation for wildlife. Generally only the most basic monitoring, if any, is done to evaluate the effectiveness of treatments and validate that objectives are being met, and information is not available to determine methods (chemical, mechanical, biological) are likely to be most effective at achieving wildlife conservation goals. Despite the widespread application of mechanical tamarisk control throughout the western United States, only limited ecologically relevant information exists regarding the effectiveness of such techniques (also see Bay and Sher 2008). It is not well understood the degree to which tamarisk removal alone may differentially direct the treated sites toward native vs. nonnative (i.e., secondary weeds) trajectories. Riparian and aquatic habitat restoration remains an inexact science, with a majority of restoration projects not meeting the goals originally envisioned (Kentula 2000, Malakoff 1998), and careful planning and adaptive management are critical to achieving policy goals (National Research Council 1992, Mitsch et al. 1998, Cairns 2000).

Thus, there is a critical need for practical information on the effects of invasive plant control and native plant restoration tools to identify the best management practices for achieving desired management goals in a cost-effective manner. In this project we sought to address such information gaps by asking the following ecosystem response questions:

- What is the capacity of native vegetation to recover following invasive plant control, and what are the mechanisms that determine the likelihood for native vs. non-native replacement vegetation?
- Will animal populations and communities respond to vegetation changes, and specifically will ‘restored’ ecosystems support new or increased use by Covered and other sensitive wildlife species?
- Can we characterize and quantify the risk status of fuels and fire regimes in restored habitats?
- Does the recent development of biological control as a tool for suppressing tamarisk fundamentally change the ecological relationships shaping vegetation and habitat patterns, and present new opportunities, and challenges, for effective rehabilitation of riparian ecosystems and associated biodiversity?

This information is specifically needed to guide the Clark County MSHCP (including the Virgin River Conservation Management Strategy), the developing Virgin River HCRP, and other management planning efforts in southern Nevada and inter-related regional riparian ecosystems. In some cases or questions the relationships between actions and responses are answered directly, while others are long-term questions that require baseline information in order to build a database for evaluating at the appropriate future date when ecosystem or biodiversity responses are realized.

Goals and Objectives of the Project

To reiterate, effective management of tamarisk and other invasive plants depends on control treatments that provide sustained weed reduction and their replacement with self-replicating native and/or beneficial non-native plants. The project Goal is to promote adaptive weed management that provides maximal, cost-effective weed control with a minimum of collateral impacts to associated beneficial species, and integrates ecosystem functions into the habitat recovery process. The overall Objective was to undertake an extensive evaluation of regional infestation sites, those where tamarisk has been treated and those where treatments were proposed, to understand the underlying interrelationships among species and environmental processes (flood and drought, physical disturbance of substrates, fire – both wild and prescribed).

In particular, the recent development of biological control measures for suppressing growth and abundance of tamarisk presents new opportunities, and challenges, for wildlife habitat management in riparian systems, and the new presence of a specialist biocontrol agent – the tamarisk leaf beetle, *Diorhabda carinulata* - in Clark County means that future management of invasive tamarisk must be conducted with the recognition that biocontrol fundamentally changes the nature of weed management processes and responses. To that end, a Supplemental project was initiated to develop the baseline information on the tamarisk biocontrol process in Clark County and adjoining regions, and a long-term monitoring program to determine how it will influence ecosystem and biodiversity processes in the future. Resulting information will be used in weed control planning to ensure that tamarisk management yields substantive restoration of native riparian wildlife habitat, and minimizes the secondary invasion by other noxious weeds that are major inhibitors of ecosystem recovery.

The specific **Objectives** of the research project were as follows:

Objective 1. Conduct a multivariate study evaluating relationships among current tamarisk dominance, dominance of other non-native and native plant species, wildlife habitat structure and wildlife associations, tamarisk control treatments, fire, and other environmental variables to assess effectiveness of weed control and riparian vegetation restoration treatments.

Objective 2. Compare vegetation structure and composition, and tamarisk flammability in relation to foliage condition, in response to experimental herbicide treatments to simulate anticipated defoliation effects of tamarisk biocontrol.

Objective 3. Compare mortality of tamarisk following fire with and without previous herbicide treatments.

Objective 4. Evaluate herbicide and cultural treatments for the control of Russian knapweed, a recent invasive species that responds positively to disturbance and may function as a surrogate for treatment of other secondary weed invasions.

Objective 5. Monitor dispersal and establishment of the tamarisk leaf beetle, document life cycles, genetic changes, and factors that may limit its establishment (predation, premature winter diapause, extreme temperatures); assess effects of this biological control agent on tamarisk condition, defoliation-re-growth dynamics and responses by associated vegetation and wildlife to tamarisk defoliation; evaluate fire risk dynamics related to defoliation.

OBJECTIVE 1. *Conduct a multivariate study evaluating relationships among current tamarisk dominance, dominance of other non-native and native plant species, wildlife habitat structure and wildlife associations, tamarisk control treatments, fire, and other environmental variables to assess effectiveness of weed control and riparian vegetation restoration treatments.*

SECTION I
AN EVALUATION OF THE EFFECTIVENESS OF TAMARISK CONTROL AS A RIPARIAN RESTORATION TREATMENT:
Case study 1 – Virgin River; Case Study 2 – Spring-fed Upland Seeps and Springs

Section Objectives

Case Study 1 -- Virgin River. The objectives of the Virgin River effectiveness monitoring study were to compare the effectiveness of tamarisk control using mechanical techniques with nearby untreated sites along the Virgin River in Nevada and Arizona. We evaluated the following hypotheses: 1.1 Tamarisk abundance is greater in untreated sites, 1.2. The abundance of non-native species is greater at treated sites, and, 1.3. Native species abundance and richness is greater at treated sites along the Virgin River.

Case Study 2 -- Spring-fed upland seeps and springs. The objectives of the spring-fed upland spring and seep effectiveness monitoring study were to evaluate the effects of tamarisk control using herbicides at select spring-fed upland seeps and spring in terms of time since initial treatment at sites located in and around Lake Mead National Recreation Area (LAKE) and the lower Colorado River within Nevada, Arizona and California. Because entire sites were treated in the past, there were no appropriate untreated areas to use as controls so we evaluated the effects of time since initial treatment. It should also be noted that although the year in which treatments were initiated at a particular site is known, it is largely unknown if the entire site was finished that year or if multiple years were required to complete treatment, plus it is unknown which sites received follow-up treatments. Thus, we were constrained to use the initial year of treatment as time zero, with the understanding that treatments and follow-up treatments may have continued for up to 5 years past this date.

Within this context, we evaluated the following hypotheses: 1) Tamarisk abundance is lowest as sites with greatest period of time since initial treatment, 2) The abundance of non-native species is greater at sites with longest period of time since initial treatment and 3) Native species abundance and richness is greater at sites with greatest time period since treatment at upland sites. Note, this supposes that total species richness will be greatest at sites with the longest period of time since initial treatment, regardless of species identity.

Materials and Methods – Case Study 1: Virgin River

Study Sites and Sampling Locations. The treated sites along the Virgin River are located approximately 14-11 km downriver (southwest) from Mesquite, NV (36°44’18.12”N 114°12’17.47”W). The untreated sites are in locations where no record of tamarisk removal or other associated disturbances were known to have occurred, but also in the vicinity of treated sites. Using Hawths analysis tools in ArcGIS 9.1 (Spatial Ecology 2011) we created a 250 m^2 grid (6.25 ha) over the treated and nearby untreated areas, resulting in 75 treatment plots (45 treated and 30 untreated). Vegetation sampling was done by establishing a 5 x 30 m vegetation plot at three randomly generated locations in the treated sites and at two random locations in the untreated sites. The number of treatment plots and number of vegetation plots were higher in the treated sites because of the higher level of variation in plant associations as compared to the untreated sites which were all dominated by tamarisk (≥70% cover). We recognize that there is a potential concern regarding sampling pseudoreplication, but we were spatially constrained to this location where the Bureau of Land Management (BLM) physically removed saltcedar, limiting the capacity to retrospectively evaluate the effects of control actions on this river system. The very large extent of the site encompassing a variety of micro-habitat sites partially compensates for the design constraints.

Tamarisk Removal Techniques. Information provided by the BLM Las Vegas Field Office indicated that the treated sites were cleared of all above-ground plant cover and the extracted biomass was removed off-site. These techniques were accomplished using heavy equipment (bulldozers), but the specific grade of machinery or intensity of extraction (number of dozers) had not been recorded. Initial field observations suggest that the treated sites were scraped and had clearly experienced substantial soil disturbance. These removal operations occurred between 2004 and 2007, and the precise timing of removal is unknown.

Vegetation Sampling. The vegetation sampling plots consisted of a 150 m^2 (30 m x 5 m) belt transect with six-1 m^2 sub-plots (USDI National Park Service 2001). Density, cover, and species identity of all woody perennial plants (i.e. shrubs, trees etc.) were measured in the 5 x 30 m belt transect plot. Each individual having >50% of its rooted base within the belt transect was counted. Data were recorded by species and age class and height to nearest 0.10 m. Age class of each individual was identified as either dead, immature-seedling, re-sprout, or mature-adult. Density of herbaceous plants was counted within the six-1 m^2 subplots along each of the two 30 m sides of the brush belt transect as subsamples, 6 total subplots. Cover of woody perennial plants, non-vascular plants, litter, and soil were measured by the point-intercept method, using one of the 30 m sides of the belt transect. Starting at the end of each transect and repeated every 30 cm, a 0.25 inch diameter sampling rod (a rigid plumb bob), graduated in decimeters, was lowered gently so that the sampling rod was plumb to the ground. Since the transect length is 30 m, there were 100 points from 30 to 3,000 cm. The height at which each species touched the sampling rod was recorded, tallest to shortest. If the rod failed to intercept any vegetation, the substrate was recorded (bare soil, rock, litter, course wood debris). Cover of herbaceous plants was measured using the Daubenmire method (Daubenmire 1968) where the observer visually estimated cover by all aforementioned cover types at each of the 1 m^2 sub-plots according to the following classes; 0=not present, 1=<1%, 2=1-5%, 3+5-25%, 4-=35-50%, 6=75-95% and 7=95-100% cover.

Data Analyses. Density and cover of shrubs and trees were averaged across vegetation plots within each 6.25 ha treatment plot. Density and cover of herbaceous species were averaged across subplots within vegetation plots then averaged across plots within treatment plots. We used square root transformed data for all analyses to meet assumptions for statistical comparisons, while presenting graphically as back-transformed data to provide visual clarity for interpretation. Error values are shown in figures as one Standard Error (SE), calculated from the pooled variance of the ANOVA model. This is a standard convention for presentation of ecological data as “SE is useful to assess the precision (repeatability) of an estimator" (Anderson et al. 2001). All analyses were performed in JMP 8.0.1 (SAS 2009).

To determine site diversity (alpha diversity) for each treatment condition, we calculated two species diversity indices, Shannon–Wiener and Simpson's D (Lande 1996) for all species using the point-intercept data records and for herbaceous species using the herbaceous density data records (see above). Density data was based on cover measures, not number of plants per species in plots; because individual plant size was not used in this analysis, we considered this a suitable index of richness for diversity analysis. We also calculated the Brillouin evenness index (Peet 1975). Simpson's D index represents the probability that two randomly selected individuals are of the same species. It takes into account both the number of species and their relative abundances, and more heavily weights the more abundant species. The Shannon–Weiner index is influenced by both the number of species and the evenness of species abundances; both a greater number of unique species and a more even species distribution increase this index. For both indices, larger values indicate greater diversity. The Brillouin evenness index is constrained between 0 and 1 and assumes that all species are represented within the sample; larger values suggest a more even community. Diversity and evenness indices were calculated by averaging abundance by species across plots per each treatment type (Using Species Diversity & Richness 4.1.2 Software; Seaby and Henderson 2006). We then used randomization tests to compare species diversity and evenness between vegetation types (Solow 1993). These analyses were considered significant at α 0.05 (Seaby & Henderson 2006).

Results and Interpretation – Virgin River

Visually the treated sites were markedly less vegetated compared to the treated plots, with the amount of bare ground appreciably greater in the treated plots (45% of all hits in treated plots compared to the untreated plots (5% of hits in untreated plots). This is not surprising given the short time since the most recent tamarisk removal activities occurred when all plants were completely removed.

Total tree and shrub species density was greater in the untreated plots (23.8 ±2.5 SE) compared to treated plots (15.8 ±2.1 SE) ($F_{1,73}$=10.11, P=0.0017). Total tree and shrub species cover (mean point hits per vegetation plot) was also significantly greater in the untreated plots (38.0 ± 2.2SE) compared to the treated plots (5.2 ±0.7SE) ($F_{1,73}$=69.4, P<0.0001). The most common species in the untreated plots was tamarisk (74% of total point hits in untreated plots) and second was arrowweed (*Pluchea sericea*) (20% of total hits). Other shrub and tree species recorded in the untreated plots included iodine bush (*Allenrolfea occidentalis*) (2.4% of hits), big saltbush (*Atriplex lentiformis*) (1% of hits), honey mesquite (*Prosopis glandulosa*) (0.3% of hits) and the non-native camel thorn (*Alhagi maurorum*) (0.3% of hits) In the treated plots arrowweed (*P. sericea*) was the most common (65% of total point hits in treated plots) and tamarisk was second (18% of total hits). Other shrub and tree species in the treated plots included big saltbush (*A. lentiformis*) (9% of hits), iodine bush (*A. occidentalis*) (4% of hits), narrowleaf willow (*Salix exigua*) (2% of hits) and peach thorn or wolfberry (*Lycium cooperi*) (1% of hits).

Tamarisk cover was also significantly higher in untreated plots (57.3 ±2.9SE) compared to treated plots (1.9 ±2.3SE) ($F_{1,73}$= 413.3, P<0.0001) (Fig. I.1.1A). Tamarisk density was also significantly greater in untreated (81.0±16.6) plots then treated plots (22.8 ±13.6SE) ($F_{1,73}$ =41.80 P<0.0001) (Fig. I.1.1B).

Herbaceous species density was slightly greater in the treated plots (9.3 ±2.0SE) compared to the untreated plots (8.2 ±2.4SE) (Figure I.2A), similarly mean herbaceous species cover was also greater in the treated plots (4.8 ±0.7SE) compared to the untreated plots (2.1 ±0.8SE), (Figure I.1.2B). Of the guilds recorded, only non-native annual grasses (NNANNGRA) had higher density in the untreated plots ($F_{1,73}$=5.64, P=0.0202) (Fig. I.1.3). Native annual forbs (NATANNFOR) ($F_{1,73}$=9.52, P=0.0029), native perennial grasses (NATPERGRA) ($F_{1,73}$=7.46, P=0.0079), non-native annual forbs (NNANNFOR) ($F_{1,73}$=17.19, P<0.0001) all had higher densities in the treated plots (Fig. I.1.3). Non-native perennial grasses (NNPERGRA) and native perennial forbs were only detected in the treated sites, although high variances among vegetation plots resulted in non-significant statistical tests (Fig. I.1.3).

Total species richness was greater in the treated plots with 52 species recorded compared to the 32 species recorded in the untreated plots (Table I.1.1). Total species and herbaceous species diversity and evenness were consistently greater in the treated sites (Table I.1.2), but again some of this is understood with increased presence and abundance of various non-native species in those plots (Table I.1.1).

In summary, it appears that vegetation in areas where tamarisk has been removed have significantly responded in various ways, but that most of this response was only detectable in areas ≥13 years after initial tamarisk treatments were applied. Treated areas displayed lower abundance of tamarisk; higher herbaceous density and cover, due primarily to native forbs, perennial grasses, and non-native annual forbs; lower density of non-native annual grasses: and higher diversity of both all species combined and herbaceous species alone. Although non-native annual grasses decreased following tamarisk removal, non-native annual forms increased. Thus, the removal of tamarisk from the Virgin River has resulted in positive effects of higher abundance of native species and higher plant species diversity, and has had mixed effects on other non-native species.

Figures and Tables

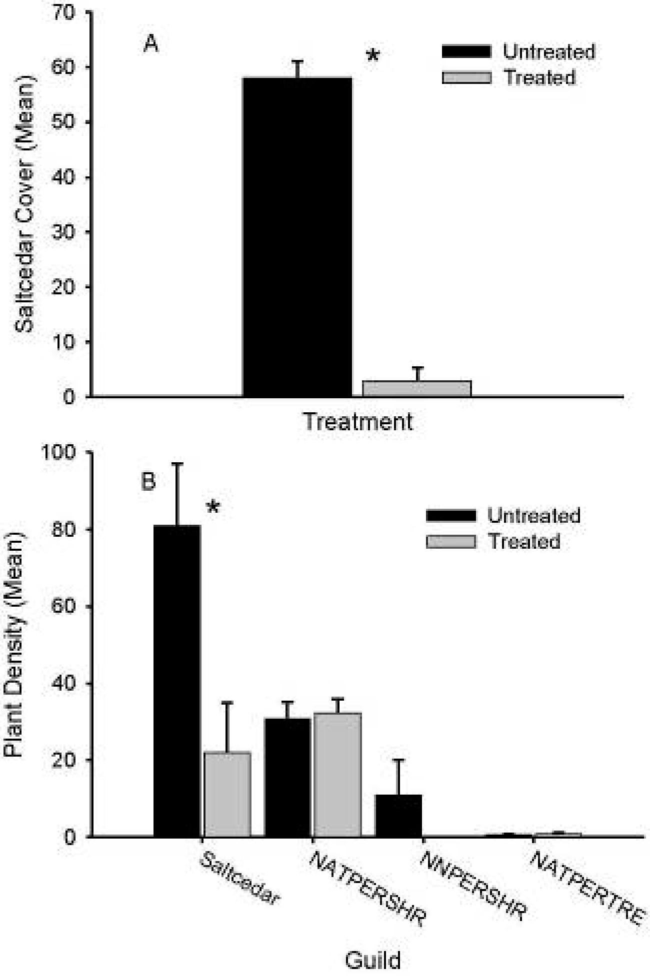


Figure I.1.1. Tamarisk mean vegetative cover (A) and mean plant density for Saltcedar (tamarisk), native perennial shrubs (NATPERSHR), non-native perennial shrubs (NNPERSHR) and native perennial trees (NATPERTRE) within 150 m^2 vegetation plots in untreated and treated sites. Comparison pairs denoted with * are significantly different, error bars signify one standard error.

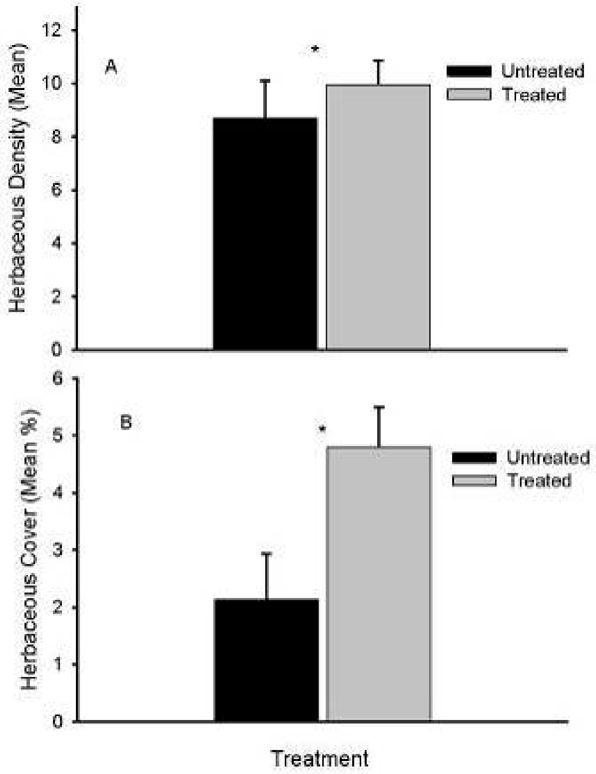


Figure I.1.2. Herbaceous species density (A) and mean relative cover (B) within 1 m^2 vegetation sub-plots in untreated and treated sites. Calculated values are based on m^2 averages within and among plots per treatment type. Comparison pairs denoted with * are significantly different; error bars signify one standard error.

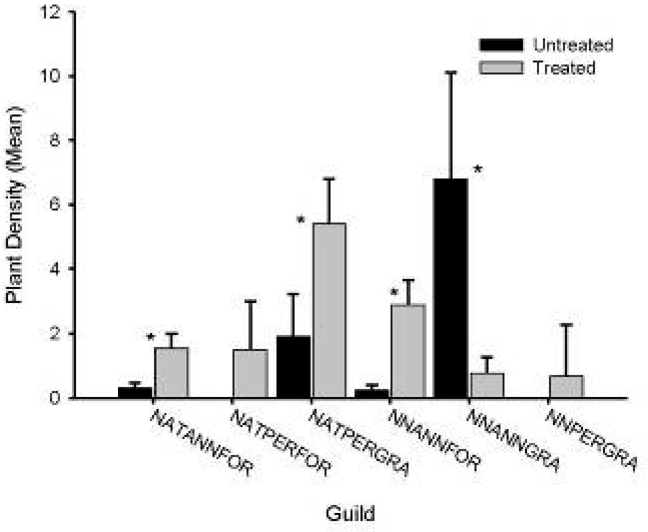


Figure I.1.3. Herbaceous species density within 1 m^2 vegetation sub-plots at by guild for the untreated and treated sites. Guilds with code ID are native annual forb (NATANNFOR), native perennial forb (NATPERFOR), native perennial grass (NATPERGRA), non-native annual forb (NNANNFOR), non-native annual grass (NNANNGRA) and non-native perennial grass (NNPERGRA). Comparison pairs denoted with * are significantly different; error bars signify one standard error.

Table I.1.1. Mean density of all shrub and tree species (A) and herbaceous species (B) that were detected in the untreated and treated plots. Guild refers to origin (NN = non-native, NAT= native)and growth form (ANN = annual, PER = perennial, SHR = woody shrub, TRE = tree, FOR = non-woody forb, GRA = grass)

A. Shrub and Tree Density				
Species	**Common Name**	**Guild**	**Untreated**	**Treated**
Alhagi pseudalhagi	camel thorn	NNPERSHR	15	0
Allenrolfea occidentalis	iodine bush	NATPERSHR	31	13
Ambrosia dumosa	burro bush	NATPERSHR	1	1
Atriplex canescens	four-winged saltbush	NATPERSHR	1	6
Atriplex hymenelytra	desert holly	NATPERSHR	0	1
Atriplex lentiformis	big saltbush	NATPERSHR	7	6
Atriplex polycarpa	cattle saltbush	NATPERSHR	0	3
Baccharis emoryi	Emory's baccharis	NATPERSHR	0	2
Baccharis salicifolia	seep willow	NATPERSHR	0	4
Baccharis sergiloides	desert baccharis	NATPERSHR	0	4
Ephedra viridis	Mormon tea	NATPERSHR	0	2
Larrea tridentata	creosote bush	NATPERSHR	1	0
Lycium andersonii	wolf berry	NATPERSHR	0	7
Lycium cooperi	peach thorn	NATPERSHR	3	16
Lycium torreyi	Torrey wolfberry	NATPERSHR	1	0
Pluchea sericea	arrow weed	NATPERSHR	467	57
Populus fremontii	Fremont cottonwood	NATPERTRE	0	1
Prosopis glandulosa var. *torreyana*	honey mesquite	NATPERTRE	2	1
Prosopis pubescens	screw bean	NATPERTRE	1	2
Salix exigua	narrowleaf willow	NATPERTRE	10	20
Salix spp.	willow	NATPERTRE	2	8
Sphaeralcea ambigua	desert globemallow	NATPERSHR	0	1
Suaeda moquinii	desert seablite	NATPERSHR	0	1
Tamarix spp.	tamarisk	NNPERTRE	27	6
Shrub and Tree Richness			**14**	**22**
B. herbaceous species density				
Species	**common name**	**Guild**	**Untreated**	**Treated**
Anemopsis californica	yerba mansa	NATPERFOR	0	2
Astragalus geyeri	Geyer's milkvetch	NATANNFOR	0	1
Bassia scoparia	burningbush	NNANNFOR	16	23
Brassica tournefortii	Asian mustard	NNANNFOR	0	2
Bromus spp.	foxtail chess	NNANNGRA	18	8
Camissonia brevipes	yellowcup	NATANNFOR	0	2

Cynodon dactylon	bermudagrass	NNPERGRA	0	14
Datura wrightii	sacred datura	NATPERFOR	0	3
Descurainia pinnata	western tansymustard	NATANNFOR	10	21
Distichlis spicata	saltgrass	NATPERGRA	33	22
Eleocharis sp.	spikerush	NATPERGRA	200	0
Eriogonum inflatum	desert trumpet	NATPERFOR	0	1
Erodium cicutarium	storksbill	NNANNFOR	16	1
Galium aparine	Gallium	NATANNFOR	1	0
Gilia transmontana	transmontane gilia	NATANNFOR	2	0
Heliotropium curassavicum	salt heliotrope	NATANNFOR	5	9
Juncus cooperi	Cooper’s rush	NATPERGRA	0	38.5
Juncus sp.	rush	NATPERGRA	0	3
Linanthus sp.	linanthus	NATANNFOR	1	0
Lotus strigosus	Strigos bird’s-foot trefoil	NATANNFOR		45
Malcomia africanus	African mustard	NNANNFOR	0	4
Phragmites australis	common reed	NATPERGRA	0	2
Poa spp.	poa	--	0	5
Polypogon monspeliensis	annual rabbitsfoot grass	NNANNGRA	167	0
Rafinesquia californica	rafinesquia	NATANNFOR	0	1
Salsola paulsenii	barbwire thistle	NNANNFOR	0	3
Salsola tragus	Russian thistle	NNANNFOR	1	10
Schismus barbatus	Mediterranean grass	NNANNGRA	33	17
Schoenoplectus pungens	common threesquare	NATPERGRA	12	29
Scirpus spp	bulrush	NATPERGRA	0	10
Sisimbrium irio	London rocket	NNANNFOR	30	3
Sisymbrium sp.	hedgemustard	NNANNFOR	3	1
Spergularia marina	salt sandspurry	NATANNFOR	0	4
Tragopogon sp.	goatsbeard	NATPERFOR	0	2
Typha latifolia	broadleaf cattail	NATPERGRA	0	2
Unknown Forbs	--	--	1	57
Unknown Grasses	--	--	0	21
Unknown Sedges	--	--	0	48
Herbaceous Species Richness			**16**	**30**
Total Richness			**30**	**52**

Table I.1.2. Simpson's D and Shannon-Wiener diversity and Brillouin evenness indices by treatment type (see Solow 1993; Magurran 2004). The treated sites were consistently greater among each diversity and evenness index based on randomization tests at a = 0.05 (Seaby and Henderson 2006).

Index	Untreated	Treated
Total Species Diversity		
Shannon Wiener	1.104	2.354*
Simpsons D	1.967	6.789*
Brillouin Evenness	0.361	0.759*
Herbaceous Species Diversity		
Shannon Wiener	1.825	2.796*
Simpsons D	4.179	13.58*
Brillouin Evenness	0.532	0.761*

Vegetation Transects

We note here that vegetation analyses were also conducted in 2009 and 2010 in collaboration with Dr. Pat Shafroth (U.S.Geological Survey, Ft. Collins CO, supported by USGS Invasive Species Program) to evaluate plant community responses to tamarisk biological control. The 24 permanent stations with cross-sectional vegetation and topographical profiles are linked with the vegetation sampling stations surveyed by Ostoja and technical assistants. They will be monitored annually beyond this project period to document the chronosequence of river segments sequentially colonized by beetles, and the changes that occur as tamarisk declines in cover and vigor, as well as how soils and stream channels respond to these changes in vegetation cover and type.

Prevalence of Cottonwood Adults and Seedling in the Virgin Valley

Riparian recovery from severe invasion by invasive plants, specifically tamarisk, is most effective when adequate supplies of native plant propagules are present for recruitment of new plants, if and when hydrological conditions are suited for dispersal and establishment. The iconic Fremont cottonwood (*Populus fremontii*) is a foundation species common in the upper portions of the Virgin watershed, but which appeared to be sparse in the Virgin Valley, with dense stands only in the vicinity of Beaver Dam Wash, Arizona, and few trees at all downstream of Mesquite, Nevada. To better understand the linkage between remaining adult trees and potential for seed dispersal and recruitment in relation to reproductive trees, we conducted a walking survey from the Virgin Gorge to the highway bridge at Riverside, NV in June 2009. Two surveyors would walk on a zig-zag path and document presence of any cottonwood plants visible, both mature trees and seedlings/juveniles, as well arboreal willows, particularly Goodding's willow (*Salix gooddingii*). Hand-held GPS units were used to log locations of individual trees, or polygons of seedling patches where densities were high, as well as height and stem diameter of plants that appeared greater than 1 or possible 2 years of age (generally >0.5 m tall). Tree species other than *Tamarix* were also recorded, including velvet ash (*Fraxinus velutinus*), desert willow (*Chilopsis linearis*), and there were also approximately 12 young Russian olive (*Eleaegnus angustifolia*) observed, most of which we hand-pulled if possible. We also noted evidence of livestock grazing based on evidence of feeding damage on cottonwood and willow plants present.

As anticipated, virtually no mature cottonwood trees and fewer than 20 mature Goodding's willows were found downstream of approximately Bunkerville, NV and those that were noted appeared to be from planted individuals rather than naturally established. Seedlings were also very sparse, but those present were closely associated with mature trees, presumably the parental sources for these plants (Fig. I.1.4). Fifty mature cottonwood trees were also selected for observing timing of reproduction in 2010, and these were monitored bi-weekly from early March until there was no evidence of further reproduction. Seed production occurred earlier in this fairly low-desert system than in some other regions, flowering starting in late February and seed release mostly being in from late March through mid-April. Seed traps were installed, but these were too late to capture the main dispersal period so were abandoned.

Based on the low dispersal distance of seedling cottonwoods, it appears that recovery of both cottonwoods and arboreal willows (which follow similar reproductive patterns ascottonwood) is unlikely to occur in the lower Virgin River as tamarisk densities decline from biological or other control factors, including flooding. Natural recovery may be more feasible in the Arizona portion of the Virgin Valley because adequate propagule supplies (mature trees) still exist, but it is likely that active restoration measures may be needed in the full portion of the River in Clark County, Nevada.

It is not clear whether the absence of these trees from the lower Valley is because environmental conditions are unsuitable, or if other anthropogenic factors may have led to low abundances. Long-term livestock grazing can cause such as situation, as cattle are very attracted to young trees of the Salicaceae, and nearly all young plants observed had experienced grazing damage (unpub data), presumably cattle since other similar browsers are not common in the area. Observations made following the 2005 El Nino flooding, cottonwood seedlings were observed in the vicinity of Riverside, but have not been seen since (Dudley, unpub data), almost certainly owing to grazing damage. Likewise, beavers tend to favor native trees for food and dam-building materials so could preferentially reduce plant survival (Mortenson et al. 2008), but their numbers were too low on the Virgin River to have caused this loss.

Thus, restoration strategies should take two major factors into account: first, that if trees are to be planted, they should be distributed in patches at distances such that subsequent seed dispersal when these trees mature will be sufficient to allow extensive recruitment with minimal area too far from adult trees for colonization; second, it may be critical to protect planted material from livestock using fencing or other devices [e.g. spine-bearing nurse plants like thorn peach or cholla cactus (*Opuntia* spp.) surrounding planted material], particularly in light of the fact that unregulated grazing continues to be a concern in much of the river system (National Park Service, pers. comm.). This is the basis for the ‘propagule island’ approach that we describe in later detail in the Discussion section of this report.

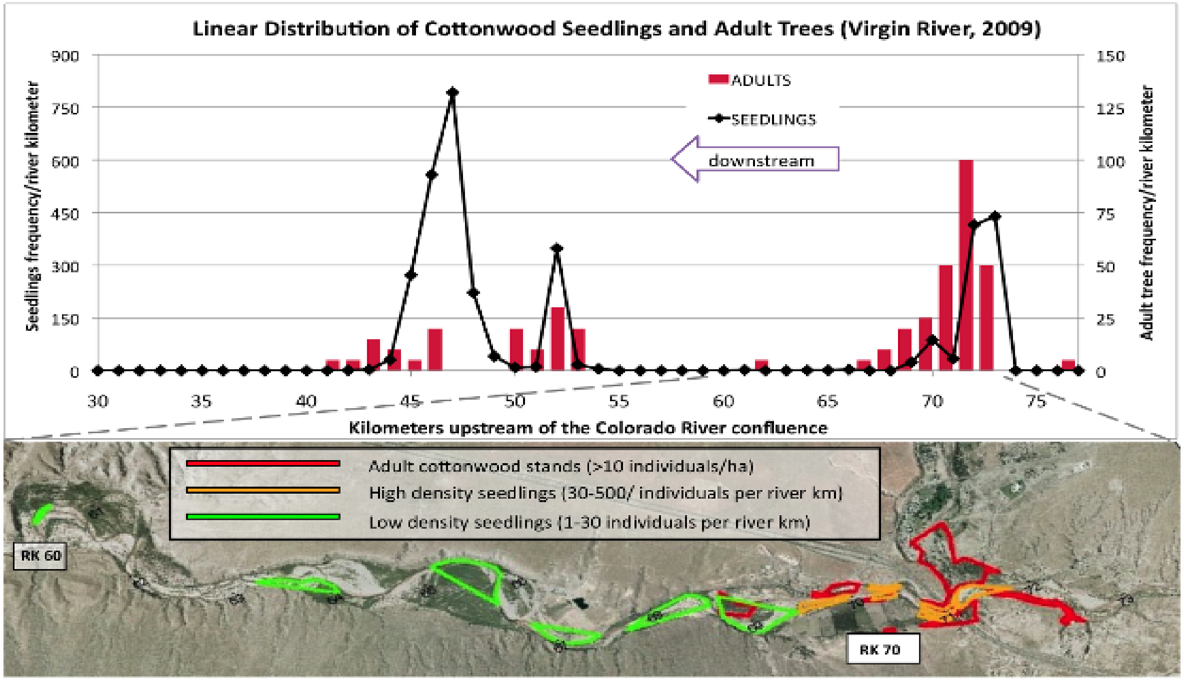


Figure I.1.4. Distribution of *P. fremontii* adult trees and seedlings along the middle segment of the Virgin River in Arizona and Nevada. Top figure shows variation in cottonwood seedling and adult abundances over a 46-km reach of the stream in summer 2009, with corresponding aerial photo (lower figure) of 10-km subreach between RK60-70 (dashed lines) showing mapped stands of adult trees and seedling patches of high and low densities extending downstream (to the left). Graphic peaks around RK70-75 are in vicinity of Beaver Dam/Littlefield AZ while peaks through RK 45-55 are found near Mesquite NV.

Materials and Methods –Case Study 2: Spring-fed upland seeps and springs

Study sites and sampling locations -The study sites were located on lands within and adjacent to Lake Mead National Recreation Area (LAKE), in AZ, CA, and NV. These sites are characterized by upland seeps and springs where tamarisk has been present. To select the specific sampling location at each site we used Hawths Analysis Tools in ArcGIS 9.1 (Spatial Ecology 2011) were we superimposed 0.25 ha grids over the treatment polygon of each treatment area. In each grid or cell we positioned a single random point where vegetation sampling was conducted. This resulted in 256 sampling locations at 22 sites (see Table I.2.1). We did not sample any untreated tamarisk dominated areas because such sites were not available. .

Tamarisk removal/control technique - Tamarisk was using the cut stump technique whereby the above ground portion of the plant is cut and removed and the stump of the severed plant is sprayed with chemical herbicide to kill the plant and prevent future re-sprouting.

Vegetation sampling design - The vegetation sampling plots consisted of a 150 m^2 (30 m x 5 m) belt transect with six 1-m^2 sub-plots. Density, cover and species identity of all woody perennial plants (i.e. shrubs, trees etc.) were measured in the 5 x 30 m belt transect plot. Each individual having >50% of its rooted base within the belt transect was counted. Data were recorded by species and age class and height to nearest 0.10 m. Age class of each individual was identified as either dead, immature-seedling, re-sprout, or mature-adult. Density of herbaceous plants was counted within the six 1-m^2 subplots along each of the two 30 m sides of the brush belt transect as subsamples, 6 total subplots. Herbaceous plants were identified to species and tallied according to guides based on life form (grass, forb), life history (annual or perennial) and whether it is native or nonnative. Cover of woody perennial, plants, non-vascular plants, litter, and soil were measured by the point-intercept method, using one of the 30 m sides of the belt transect. Starting at the end of each transect and repeated every 30 cm, a 0.25 inch diameter sampling rod (a rigid plumb bob), graduated in decimeters, was lowered gently so that the sampling rod was plumb to the ground. Since the transect length is 30 m, there were 100 points from 30 to 3,000 cm. The height at which each species touches the sampling rod was recorded, tallest to shortest. If the rod failed to intercept any vegetation, the substrate was recorded (bare soil, rock, litter, course wood debris). Cover of herbaceous plants was measured using the Daubenmire method (Daubenmire 1968) where the observer visually estimated cover by all aforementioned cover types at each of the 1 m^2 sub-plots according to the following classes; 0=not present, 1=<1%, 2=1-5%, 3+5-25%, 4-=35-50%, 6=75-95% and 7=95-100% cover.

Data analyses - Density and cover of shrubs and trees were averaged across plots per site, we compared sites in terms of tamarisk density and cover, total species richness, native and non-native species density, cover and richness using ANOVA by considering treatment year (years since treatment) was the predictor variable. Time since treatment was considered according to categorical relative groupings where we combined sites treated prior to sampling in the following three categories; 2-6 years, 7-12 years and ≥13 years since initial treatment year. When significant differences were found we conducted a Tukey-HSD test to determine which of the groups differed (Zar 2009). All statistics and analyses were performed in JMP 8.0.1. We used square root transformed data for all analyses to meet assumptions for statistical comparisons but present back-transformed data visually in figures. All analyses were performed in JMP 8.0.1 (SAS 2009).

We constructed Rényi curves (Rényi 1961) for interpretation of diversity patterns among the treatment histories. This method of diversity ordering allows for the interpretation of diversity patterns across a range of indices by plotting index values against the scale parameter to determine whether species diversity may unambiguously differ between treatment periods (see Rényi 1961, Hill 1973). Larger Rényi index values (y-axis) suggest greater levels of species diversity at a given point along the scale parameter (x-axis). The scale parameter represents a range of diversity metrics that differ in their sensitivity to abundant species; lower (0, 1) scale parameter values give less weight to abundant species than do larger scale parameter values (3,4). All diversity and/or evenness calculations and comparisons were done in Species Diversity and Richness 3.03 (Using Species Diversity & Richness 4.1.2 Software, Seaby and Henderson 2006).

Results and Interpretations – Spring-fed upland seeps and springs

Tamarisk density was significantly greater at sites treated within six years prior to sampling compared to sites where treatments occurred seven or more years prior to sampling ($F_{2,253} = 13.18$, $P < 0.0001$) (Fig. I.2.1A). This pattern may be best understood in light of how the National Park Service Lake Mead Exotic Plant Management Team (EPMT) actively monitors and manages each site The EPMT team revisits and reassesses previously treated areas to determine if retreatments are needed. Retreatments target previously untreated or surviving tamarisk plants to increase the likelihood of total site eradication. In many cases retreatments do not occur for many years past initial treatments. We surmise that many of the sites that were treated <6 year prior to our vegetation sampling have not yet been revisited and retreated by the EPMT team, explaining the higher density of tamarisk at those sites compared to sites treated further in the past.. Native shrub density did not differ significantly among years since initial treatment ($F_{2,253} = 2.02$, $P=0.1336$) (Fig. I.2.1B). In contrast , native tree density was highest at sites with the longest time since initial treatment ($F_{2,253} =13.85$, $P < 0.0001$) (Figure I.2.1C). Although statistically significant, it is debatable whether 5 native trees per 150 m^2 vegetation plots in areas initially treated ≥13 ago is ecologically different from 2 native trees per plots initially treated ≤12 years ago (Fig. I.2.1C). .

Herbaceous species density did not differ among the years since initial treatment groupings ($F_{2,253} =2.11$ $P<0.1232$) (Fig. I.2.2A). In contrast, herbaceous species cover was higher at sites that have the longest time since treatment ($F_{2,253} =24.36$ $P<0.0001$) (Fig. I.2.2B). Again, although statistically significant, the difference between 7% cover in areas initially treated ≥13 ago may not be ecologically different from 3-4 % cover in areas initially treated ≤12 years ago (Fig. I.2.2B). When considering species density by guild; we found that native annual forbs ($F_{2,253} =9.96$ $P<0.0001$), native annual grasses ($F_{2,253} =13.56$ $P<0.0005$), native perennial forbs ($F_{2,253} =15.52$ $P<0.0001$), and native perennial grasses ($F_{2,253} =5.08$ $P=0.0072$) where all greater in the sites with the longest time since initial treatment Fig. I.2.3). In contrast, non-native annual forbs, non-native annual grasses, and non-native perennial grasses did not significantly differ among years since initial treatment. Species diversity was lowest at sites with the shortest (<6 yrs) years since initial treatment for woody perennial species (Fig. I.2.4A) as well as for herbaceous species (Fig. I.2.4B).

In summary, it appears that vegetation in areas where tamarisk has been removed have significantly responded in various ways, but that most of this response was only detectable in areas ≥13 years after initial tamarisk treatments were applied. Treated areas displayed lower abundance of tamarisk and higher abundance of native trees; higher herbaceous cover, due primarily to native annual and perennial forbs and grasses; and higher diversity of both woody and herbaceous species. Non-native forbs, annual grasses, and perennial grasses did not appear to have responded one way or another to tamarisk removal. Thus, the removal of tamarisk from upland springs and seeps in the Mojave Desert has resulted in higher abundance of native species and higher overall plant species diversity, but this result only became apparent slightly over a decade after initial treatments were applied.

Figures and Tables, Section I.2

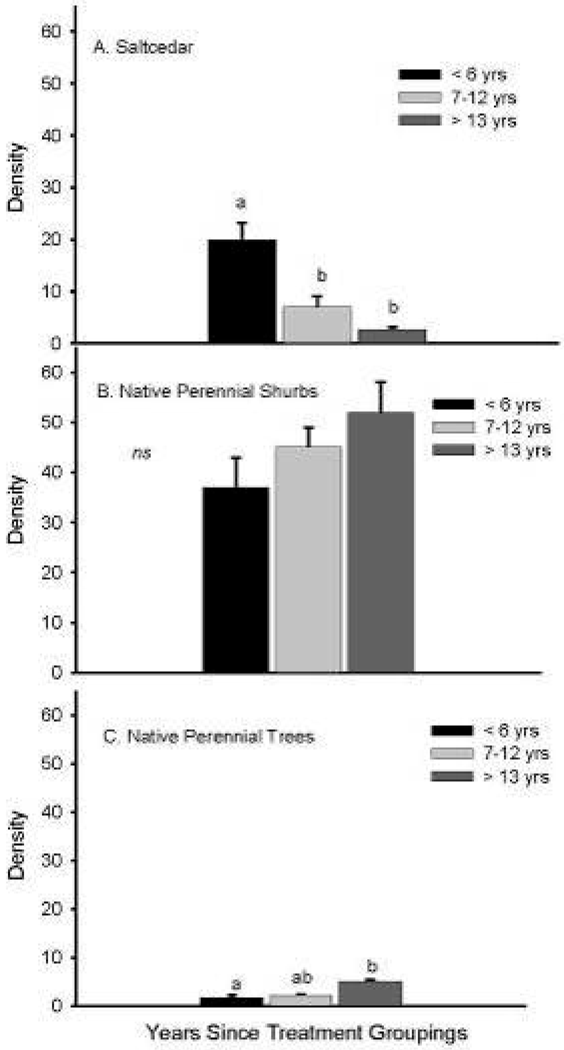


Figure I.2.1. Tamarisk density (A), native perennial shrub density (B) and native tree density (C) within 150 m^2 vegetation plots at spring- fed sites at or around Lake Mead National Recreation Area at sites that were treated between 2 and 18 years prior to sampling and depicted here according to categorical groupings of sites that were treated in one of three periods relative to 2009, the year sampling was conducted. Groups with different letters are significantly different at α 0.05 while *ns* indicated that comparisons were not significant; error bars signify one standard error.

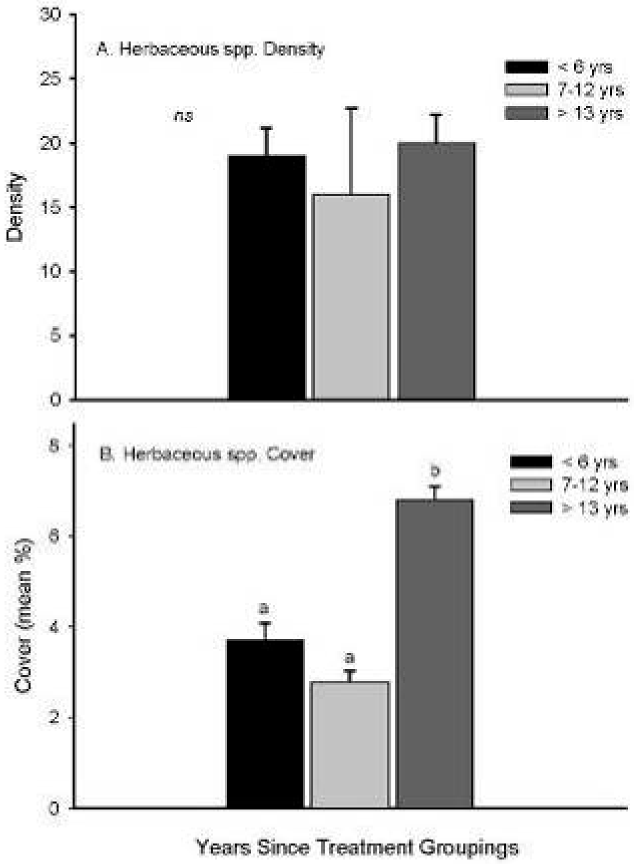


Figure I.2.2. Herbaceous species density (A) and mean (%) cover (B) within 1 m^2 vegetation sub-plots at spring-fed sites at or around Lake Mead National Recreation Area at sites that were treated between 2 and 18 years prior to sampling and presented here according to categorical groupings of sties that were treated in one of three periods relative to sampling in 2009. Groups with different letter are significantly different at α 0.05; error bars signify one standard error.

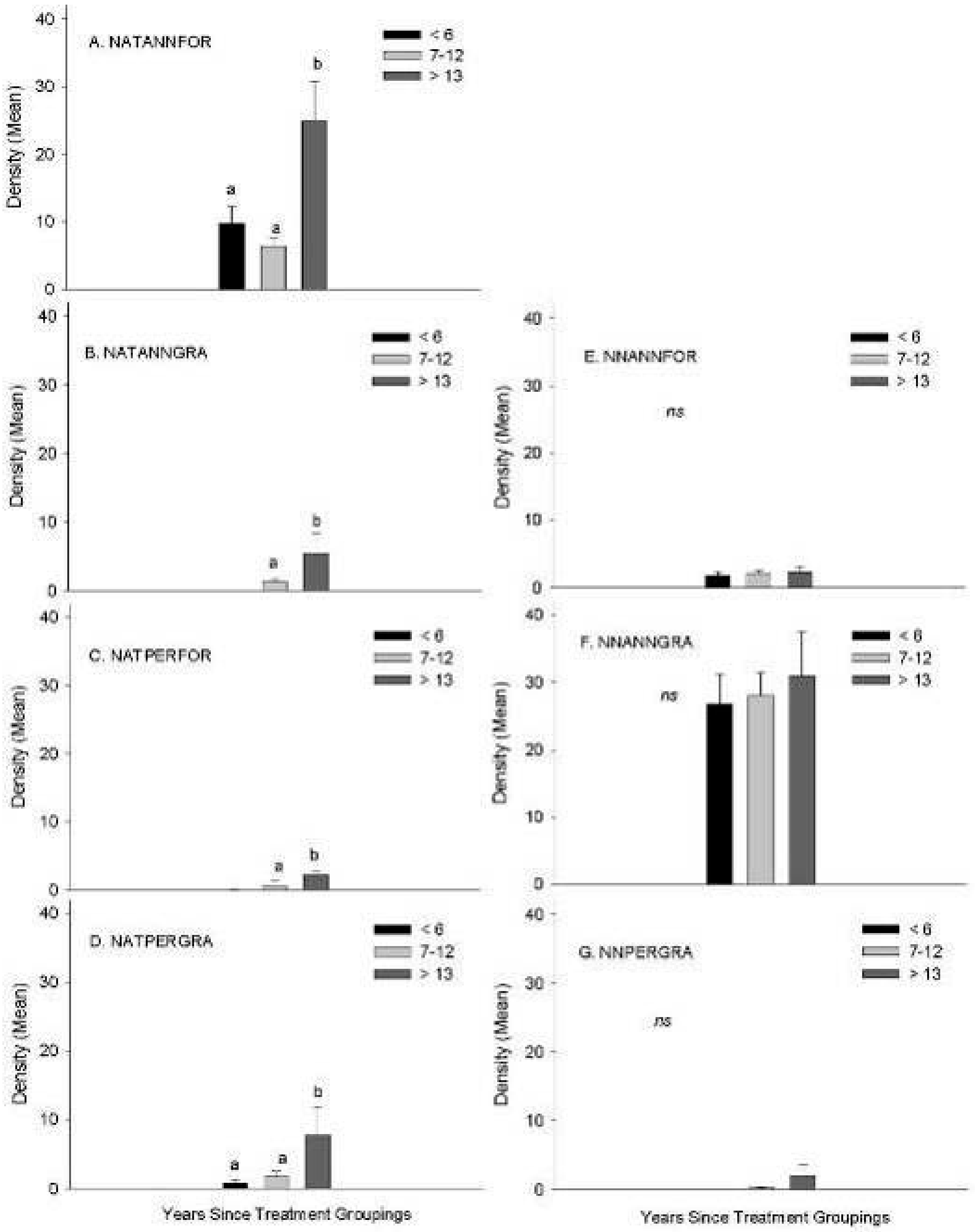


Figure I.2.3. Herbaceous species density (1 m^2) by guild within 1 m^2 vegetation sub-plots at the spring-fed seep and spring upland sites at or around Lake Mead National Recreation Area at sites that were treated between 2 and 18 prior to sampling in 2009 and presented here according to categorical groupings of sites that were treated in one of three periods prior to the sampling year. The guilds and codes are shown in (A) native annual forbs (NATANNFOR), (B) are native annual grasses (NATANNGRA), (C) are native perennial forbs (NATPERFOR), (D) are native perennial grasses (NATPERGRA), (E) are nonnative annual forbs (NNANNFOR), (F) are nonnative annual grasses (NNANNGRA) and (G) are nonnative perennial grasses (NNPERGRA). Groups with different letter are significantly different at α 0.05 as within group comparisons only; error bars signify one standard error.

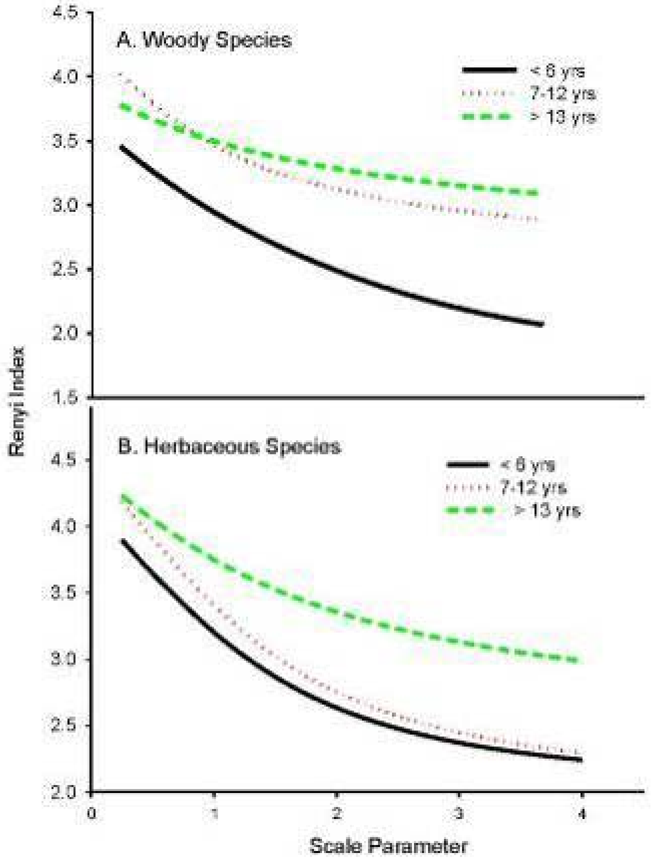


Figure I.2.4. Renyi diversity ordering for perennial woody species (A) and herbaceous species at or around Lake Mead National Recreation Area at sites that were treated between 2 and 18 prior to sampling in 2009 and presented here according to categorical groupings of sites that were treated in one of three periods prior to the sampling year. Lines that are either consistently above or below one another demonstrate greater or lower species diversity however lines that cross along the x-axis suggest differences in species diversity among respective groups cannot be concluded.

Table I.2.1. The site names and number of plots sampled as part of the upland spring-fed effectiveness monitoring sampling effort.

Site	# Plots	Treatment Year	Avg. Precip (cm)	UTM East/North
Agua Chiquita	4	2007	6.56	742552/4014894
Blue Point Springs	15	1992	6.56	730319/4030243
Cataract	10	2005	6.56	745425/4011718
Catclaw	5	1999	6.56	739816/4014516
Echo Wash	28	1999	6.56	723403/4018446
Fire Cove	14	2006	6.56	732705/4031379
Fire Wash	13	2001	6.56	731181/4032271
Fish Hatchery	15	2006	5.11	696529/3995219
Gold Strike	10	2006	5.11	703387/3986131
Grapevine Canyon	24	1993	5.46	713285/3900835
Gypsum Wash	29	2000	6.56	689147/4008650
Hiko Canyon	3	1999	5.46	711565/3894330
Meadow Valley	4	2001	6.56	730903/4029139
Picture Canyon	8	2003	5.46	707200/3882434
Pigeon Wash	6	2005	6.84	236139/4017040
Pipe Springs	10	2002	5.46	711086/3906602
Red Bluff	16	2002	6.56	746202/4038575
Roger Springs	7	2000	6.56	756306/4075199
Sacatone Canyon	9	1991	5.46	711934/3902269
Sugar Loaf	6	1997	5.11	703849/3986323
Tassi Wash	6	1999	5.11	236079/4016196
Vegas Wash	13	1996	5.11	688186/3999278

SECTION II (OBJECTIVE 1)
AVIAN RESPONSE TO TAMARISK REMOVAL ON THE VIRGIN RIVER, NEVADA

Background and Section Objectives

As one of the most successful plant invaders in the western United States, tamarisk has a reputation for impacting ecosystem structure and function (Hultine et al. 2010) and reducing biodiversity habitat quality (Bailey et al. 2001; Bateman et al. 2010). Ellis (1995), on the other hand, reported that while bird species composition differed, total species richness was similar among native cottonwood and tamarisk (*T. chinensis*) sites. The mechanical and chemical control measures generally used on large tamarisk stands by land management agencies potentially impact soils, vegetation and subsequent habitat quality for wildlife in both the short- and long-term. However, ecologically meaningful studies evaluating the effects of such actions are lacking.

In the southwestern United States, riparian habitat quality is critical to numerous breeding, migrating, and wintering birds (Anderson & Ohmart 1977; Knopf et al. 1988; Paxton et al. 2008). Compared to the adjacent uplands, these essential riparian habitats support 10 times more birds in a given year (Stevens et al. 1977; Skagen et al. 1998; Anderson et al. 2004). While much emphasis has been focused on the southwestern willow flycatcher, avian community responses to tamarisk dominance and habitat quality suggest mixed results. Several studies have shown that vegetation species composition influenced bird abundance and diversity (Anderson & Ohmart 1977; Rice et al. 1984; Hunter et al. 1988) while other research suggested that habitat structure, rather than composition, had a stronger influence on bird communities (Sogge et al. 2005). Despite conflicting results and the unresolved debate on the habitat quality of tamarisk compared to native vegetation, birds will use and breed successfully in tamarisk (Brown et al. 1987, Hunter et al. 1988, Fleishman et al. 2003, Sogge 2005). Consistently, research indicates that some vegetation structure is better than no structure such as what often results from tamarisk removal programs that lack post-removal re-vegetation and restoration applications.

We evaluated the short-term response of avian communities along the Virgin River in southern NV to mechanical control treatments. The Bureau of Land Management (BLM) employed heavy equipment to remove all above-ground vegetation down to the mineral soil. Our hypotheses comparing conditions in untreated and treated areas during the first few years post-treatment (2-4 years) were as follows: 1) bird abundance will be higher in untreated than treated sites, 2) bird species diversity will be higher in untreated than treated sites, and 3) overall patterns of bird abundance and diversity will be positively associated with corresponding metrics reflecting habitat complexity and structure.

Methods

Study Area

The study area was located in the eastern Mojave Desert along the Virgin River in southern Nevada. It encompassed 34 river km and followed the river corridor between 36°48’2.60”N 114°3’0.09”W at 1590’ elevation (adjacent to Mesquite, NV) and 36°38’22.59”N 114°18’59.86”W at 1260’ elevation (near Overton, NV) (Fig. II.1). The BLM treated many sites within the study area to remove tamarisk using a variety of mechanical techniques 2-4 years (2005-2007) prior to our field data collection. Unfortunately, BLM records lacked specific details describing the types and seasonal applications of their treatment methods. Without this information, we were unable to determine if treatment technique or time since treatment influenced the avian community or habitat variables. In addition, initial cursory observations of the study areas suggested that there was a lot of variation in vegetation characteristics among the treated sites. Therefore, we established as many treated plots as possible to account for this high variation, although we were limited by our relatively large plot size and the finite area where BLM removed tamarisk.

At the time of data collection, the tamarisk leaf beetle (*Diorhabda carinulata)* introduced throughout for the biological control of tamarisk was >50 river km away from our closest untreated plots. We never observed any *Diorhabda* beetles in any of our plots throughout our entire sampling period. Thus, the short-term results of this study should be interpreted within the context of a southwestern riparian area that has not yet been colonized by this biocontrol beetle.

Field Methods

Using Hawth's analysis tools in ArcGIS 9.1 (Spatial Ecology 2011), we overlayed a 250 m x 250 m grid (6.25 ha) onto a map of treated and untreated areas and identified 30 untreated plots and 39 treated plots (Fig. II.1). We located untreated plots adjacent to the treated plots in areas where we had no record of tamarisk removal or other associated disturbances. We clearly marked all plot boundaries with flagging tape during March 2009, before the nesting season, and maintained those markers throughout the surveying season. We also used rebar to permanently mark the plot corners in case site disturbance removed large numbers of our flagging.

We used spot mapping techniques (Ralph et al. 1993) to survey the avian community in the plots with eight surveys of each plot during the breeding season (April – July 2009). Before we started surveying for birds, all surveyors had to pass a test, given by external experts, of bird species identification abilities for both visual and auditory cues of all birds (resident or migrant) known to occur in our study area. We started surveying at dawn and ended no later than 11:00 AM after which time bird activity dramatically declined throughout the study area (S. Roberts and C. Grattan pers. obs.). To ensure we surveyed each plot during the same stage of the breeding season, we randomly assigned survey times to each plot and surveyed all 69 plots within 10 days during each of the 8 survey periods. During each survey, an observer slowly walked in a random pattern through the entire plot for 1-2 hours depending on the vegetation thickness. We used Garmin hand-held global positioning systems to map all bird locations directly onto a recent enlarged digital orthophotograph of the plot. For each bird observation, we also recorded species, sex, age, behavior (calling, singing, counter-singing, etc), and bird location accuracy onto the orthophotograph. We digitized all bird locations into ArcGIS (ESRI v. $9^{©}$) and a single analyst delineated all bird territories using ArcMap to simultaneously view all bird locations in a plot for each species. We then tallied the number of individuals we observed in each plot, using the delineated territories to ensure subsequent observations of individual birds did not result in double-counting.

We conducted vegetation sampling within each of the bird survey plots by establishing a 5 m x 30 m vegetation sub-plot at three randomly generated locations within the treated plots and at two random locations in the untreated plots. We increased the vegetation sampling effort in the treated plots due to the higher level of spatial variation in plant associations compared to the untreated plots which had a fairly uniform ≥70% overstory cover of tamarisk. We recorded the following habitat variables within each vegetation sub-plot: 1) tamarisk stem density for mature and immature tamarisk, 2) native tree and shrub stem density, 3) vegetation species richness (10m x 30m scale), 4) herbaceous species density, 5) percent vertical vegetation cover (physiognomic complexity), and 6) treatment condition (treated or untreated).

We measured physiognomic complexity using a Nudds board (Nudds 1977). Our Nudds boards were 30 cm x 250 cm shelving boards with the long length painted in a repeating pattern of white then black in 50 cm segments. Nudds cover field sampling consisted of one person holding the board at distances of 5 m and 15 m away from an observer at four fixed locations within each plot. At each of the four locations and for each distance, the observer recorded the percent of each segment that was obstructed by vegetation. This resulted in a quantitative ocular measure of board segment cover (%). We then averaged the five Nudds cover values point^{-1} and subsequently averaged the four 5 m and 15 m distance readings plot^{-1} for a single mean Nudds cover value for each plot.

Data Analyses

To investigate the effects of tamarisk removal on avifauna at the community level, we used non-metric multidimensional scaling (NMDS) to assess how the birds "map" themselves onto the habitat in treated and untreated areas. The NMDS uses the rank order of Sørenson distances (Sørenson 1948, Bray and Curtis 1957) to establish similarities in bird abundances among the 69 plots. Theoretically, the birds closest together on the ordination share the most similarities in habitat associations. We also used NMDS to evaluate the dissimilarities between habitat variables measured at each plot and overlayed that on top of the bird ordination. Similarly for the habitat data, the plots closest together in NMDS space share the most similarities in the measured habitat variables. The axes of the NMDS ordination represent gradients that are a composite function of the variables. The dissimilarity between the distribution of data points in the original data space and the reduced ordination data space is quantified as "stress," which scales from 0 to 100 (McCune and Grace 2002). The lower this dissimilarity, the lower the stress and the better the "fit". We selected the dimensionality of the NMDS ordination using scree plots, which depict how stress decreases with increasing dimensions. We used Pearson's coefficient of determination to quantify the proportion of variation (fit between the original data space and the distance in the ordination) represented by each composite axis. To reduce skew, we transformed the data using either arcsine-square root (physiognomic complexity data) or $\log_{10}$ (y+1) for abundance and density variables. To limit the number of zeros in the data matrix, we excluded rare bird species (i.e., <5 individuals observed or observed on <3 plots) from analyses (Legendre & Legendre 1983, Brazner & Beals 1997); this left 57 (out of 81) bird species for the ordination with the six habitat variables. We also removed all water-dependent species (mallard, American coot, American bittern, black-necked stilt, great blue heron, and green-winged teal) because plot location in reference to water would confound the presence of these species.

To assess the effects of tamarisk removal on avian foraging guilds, we assigned each species to its corresponding foraging guild according to Ehrlich et al. (1998). We only included the six foraging guilds (ground glean, foliage glean, hover and glean, aerial foraging, swoops, and hawks) that corresponded to the same 57 species we used in the abundance ordination. We used NMDS to determine if tamarisk removal influenced bird spatial distribution in terms of their primary foraging behavior.

Due to the regional interest in the effects of tamarisk removal on the Clark County Covered Species and bird species of special conservation concern, we tested for treatment effects on all of the covered and special status species we observed during our surveys. We also included three other species that are known to show sensitivity to changes in habitat quality. We observed >2 occurrences of the following ten bird species of special conservation concern: Abert's towhee (ABTO), Arizona Bell's vireo (BEVI), blue grosbeak (BLGR), Crissal thrasher (CRTH), loggerhead shrike (LOSH), Lucy's warbler (LUWA), phainopepla (PHAI), southwestern willow flycatcher (WIFL), yellow-breasted chat, (YBCH), and yellow warbler (YWAR). By only including species with occurrences of ≥3 individuals, we avoided generating spurious results (McCune and Grace 2002). We again used nonparametric tests, multi-response permutation procedure (MRPP) and indicator species analysis (Zar 2009), to evaluate whether tamarisk removal affected these covered species as a group and as individual species, respectively. This is also the minimum number (3) of sightings necessary to validate presence of the endangered southwestern sub-species of willow flycatcher. Community data often break the rigid assumptions of parametric statistics (e.g. normality and homoscedasticity) and nonparametric methods do not require these distributional assumptions. For consistency in our analyses, we again used Sørenson distances (Sørenson 1948, Bray and Curtis 1957) to examine the difference in covered bird species density as a group between treated and untreated plots (MRPP). To evaluate the differences in individual covered species due to tamarisk removal, we performed 5000 permutations in the Monte Carlo test (Indicator Species Analysis). We used PC-ORD (MjM software design, http://home.centurytel.net/~mjm/) to perform the NMDS, MRPP, and indicator species analysis according to guidelines outlined by McCune and Grace (2002).

To determine site diversity (alpha diversity) for each treatment condition, we calculated two species diversity indices, Simpon's index of diversity (1-D) and Shannon-Weiner (H') (Lande 1996). Species evenness is a measure of the relative abundance of all of the different species compiling the richness of an area. Increases in species richness and evenness leads to increases in diversity and both of these indices incorporate both richness and evenness. Whereas the Simpon's index heavily weights the more abundant species (Magurran 2004), the Shannon-Weiner index is more sensitive to rare species. For both of these indices, higher index values correspond to more diverse communities. We used analysis of variance (ANOVA) to compare species diversity and evenness between the treatment conditions and used $\alpha = 0.05$ for all statistical tests. We used program JMP 8.0.2 (SAS 2009) to conduct these ANOVAs.

Results and Interpretation

Overall bird abundance was higher in untreated plots (18 individuals ha^{-1} ±3 SE) compared to treated plots (6 individuals ha^{-1} ±1 SE) ($F_{1,68} = 82.18$, $p < 0.0001$) 2-4 years post-treatment. The NMDS results also illustrate that the abundances of the majority of bird species were higher in untreated than treated plots. Most of the points representing bird species abundance (Fig. II.2, black stars) were clustered within the same ordination space as that occupied by the untreated sites (Fig. II.2, shaded circles). Axis 2 of the ordination indicates that physiognomic complexity ($r^2 = 0.63$) and tamarisk density ($r^2 = 0.50$) were uniformly high for untreated plots but highly variable among treated plots. Although there was higher variability in physiognomic complexity in the treated plots, nearly all of the treated plots had substantially lower complexity than all of the untreated plots (Fig. II.2, open circles). With the exception of one outlier, the variability in physiognomic complexity and tamarisk density in the treated plots ranged from low to lower. The consistently low physiognomic complexity in the treated plots indicates that the vegetation at the treated plots lacked vertical and horizontal structure.

The foraging guild NMDS illustrates how foraging behavior can influence avian habitat use (Fig. II.3). Assuming the insects, seeds, fruits, and leaves consumed by the foliage and ground gleaning birds are positively associated with vegetation density, those foraging guilds would be also be associated with the untreated plots with high vegetation density. The converse is true for birds needing more open space to pursue and capture their prey as is evident by the swooping, hawking, and aerial foraging birds being associated with more open habitat (Fig. II.3). These results suggest that to maximize diversity in avian foraging guilds, it is essential to maintain habitat heterogeneity across the landscape scale consistently through time. This could be accomplished by alternating tamarisk removal areas with untreated sites, although this approach would reduce the effectiveness of removal effort by leaving tamarisk seed sources nearby that could promote recolonization. Alternatively, post-treatment revegetation of native trees and shrubs could be applied in a landscape patchwork to promote habitat heterogeneity.

The Simpson's index representing avian species diversity was significantly higher in the untreated plots (0.92 ±0.01) than the treated plots (0.87 ±0.02) ($F_{1,68}$= 27.42, p <0.0001). The Shannon-Weiner diversity index was also significantly higher in the untreated plots (2.82 ±0.06) than in the treated plots (2.33 ±0.12) ($F_{1,68}$= 53.51, p <0.0001).

Very few avian species were more abundant in the treated plots compared to the untreated plots, and the abundances of many species were too low to evaluate individually (Table II.2). Focusing on the most common species, plus seven others that have special conservation status in or near the study area (Tables II.3, II.4), we used a histogram to illustrate the differences in bird densities within each treatment type (Fig. II.4). As a group, the ten avian species with special conservation status that we tested were significantly different in their abundances in the untreated compared to treated plots with A= 0.31 and p < 0.0001) (Table II.1 and Fig. II.4). On an individual species basis, all but the loggerhead shrike and phainopepla were significantly more abundant in the untreated plots. Although we observed a slightly higher abundance of phainopepla in the untreated plots, the difference was not large enough to warrant statistical significance (p=0.30) as is also apparent by the overlapping error bars in the histogram. The loggerhead shrike was significantly more abundant in the treated plots (p=0.0018) possibly due in part to its foraging behavior of swooping in on its prey, and therefore needs open space to forage.

The eight species that we tested that were more abundant in the untreated plots could be responding to the increased cover and physiognomic complexity provided in the untreated plots for a variety of reasons, including increased protection and/or increased food. An important point to note is that the parasitic brown-headed cow bird was nearly ten times more abundant in the untreated plots (Fig. II.4 and Table II.2) suggesting that nest parasitism could be a conservation concern for special status species using the dense tamarisk for nesting. Spatially and temporally breaking up the landscape with areas in a variety of different post-removal and restoration stages could help minimize nest parasitism by cow birds. Finally, with regard to concerns that tamarisk removal hinders willow flycatcher (WIFL) population persistence, we observed WIFL (n=4) only in untreated plots. However, two of the four individuals that we observed were in plots with native willow (*Salix* sp.) stands mixed in among the tamarisk (also see Appendix I of this report).

It is important to clarify that surveys were restricted to areas that had been treated to reduce tamarisk dominance within the previous 5 years (and particularly in light of major flooding impacts to this riparian zone during 2005. Thus, it was not feasible to assess whether a longer period of time post-treatment could have allowed sufficient time for re-establishment of native vegetation which would likely have supported a richer and more abundant native avifauna. However, parallel vegetation assessment suggests that, at least in this region, it may be difficult to facilitate recovery of native vegetative assemblages without management intervention by active re-vegetation (Section I, this report).

Figures

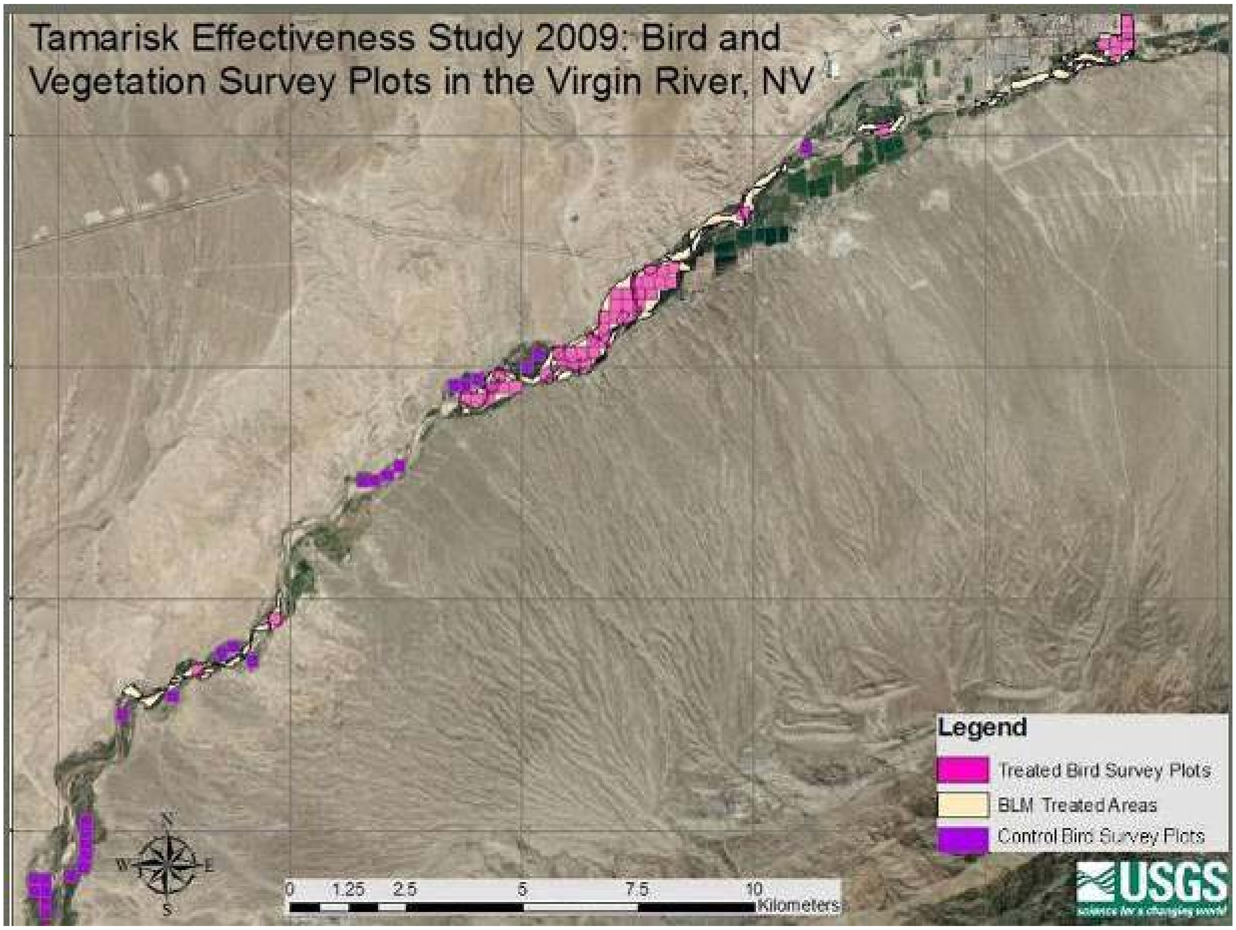


Figure II.1. Locations of the 6.25 ha (250 m x 250 m) bird survey plots along the Virgin River between Mesquite, NV and Overton, NV.

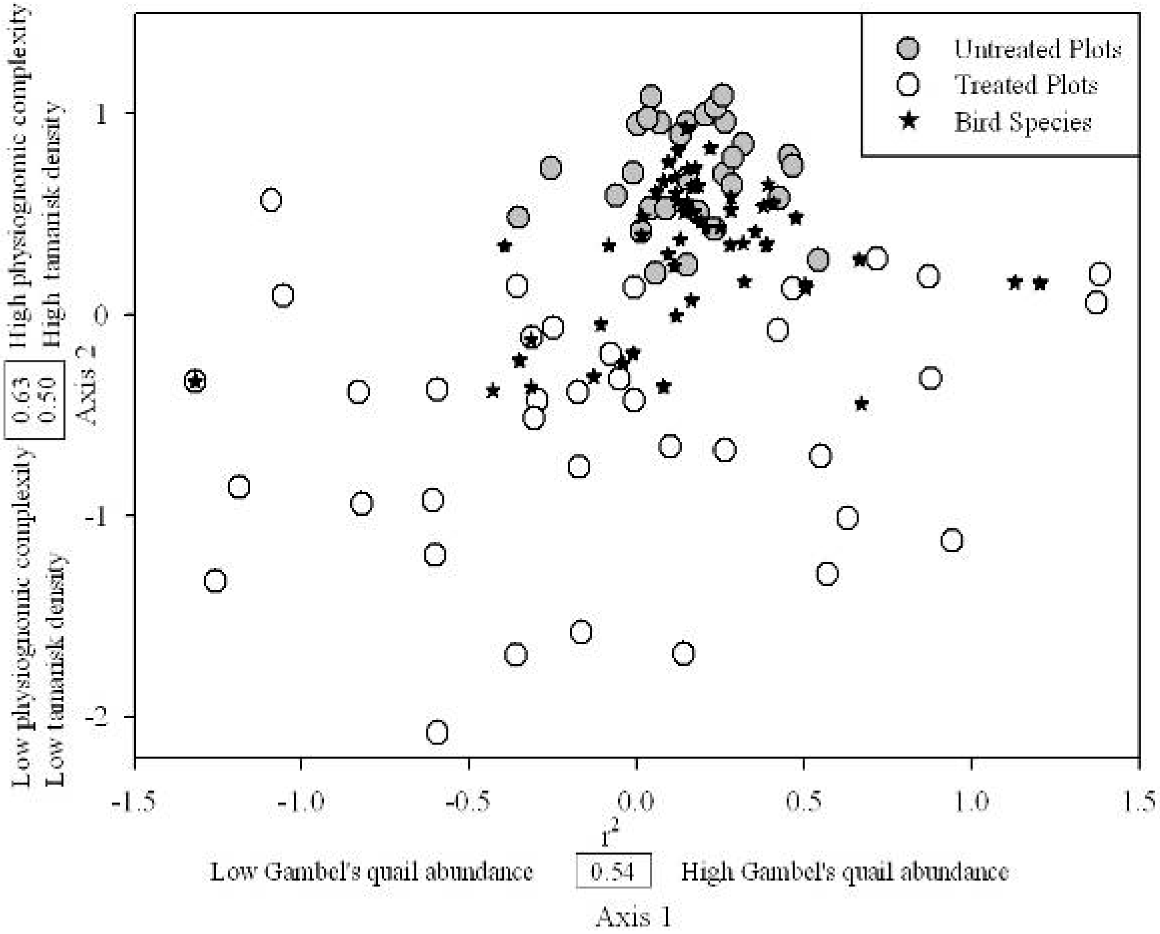


Figure II.2. Non-metric multidimensional scaling ordination for the abundance of 57 different bird species and six habitat metrics collected at the 69 bird spot mapping plots along the Virgin River, NV. The untreated plots (n=30) had >70% cover of tamarisk while the treated plots (n=39) had tamarisk mechanically removed 2-4 years prior to our surveys. The variables with a coefficient of determination (r^2) of >0.5 are listed on each axis.

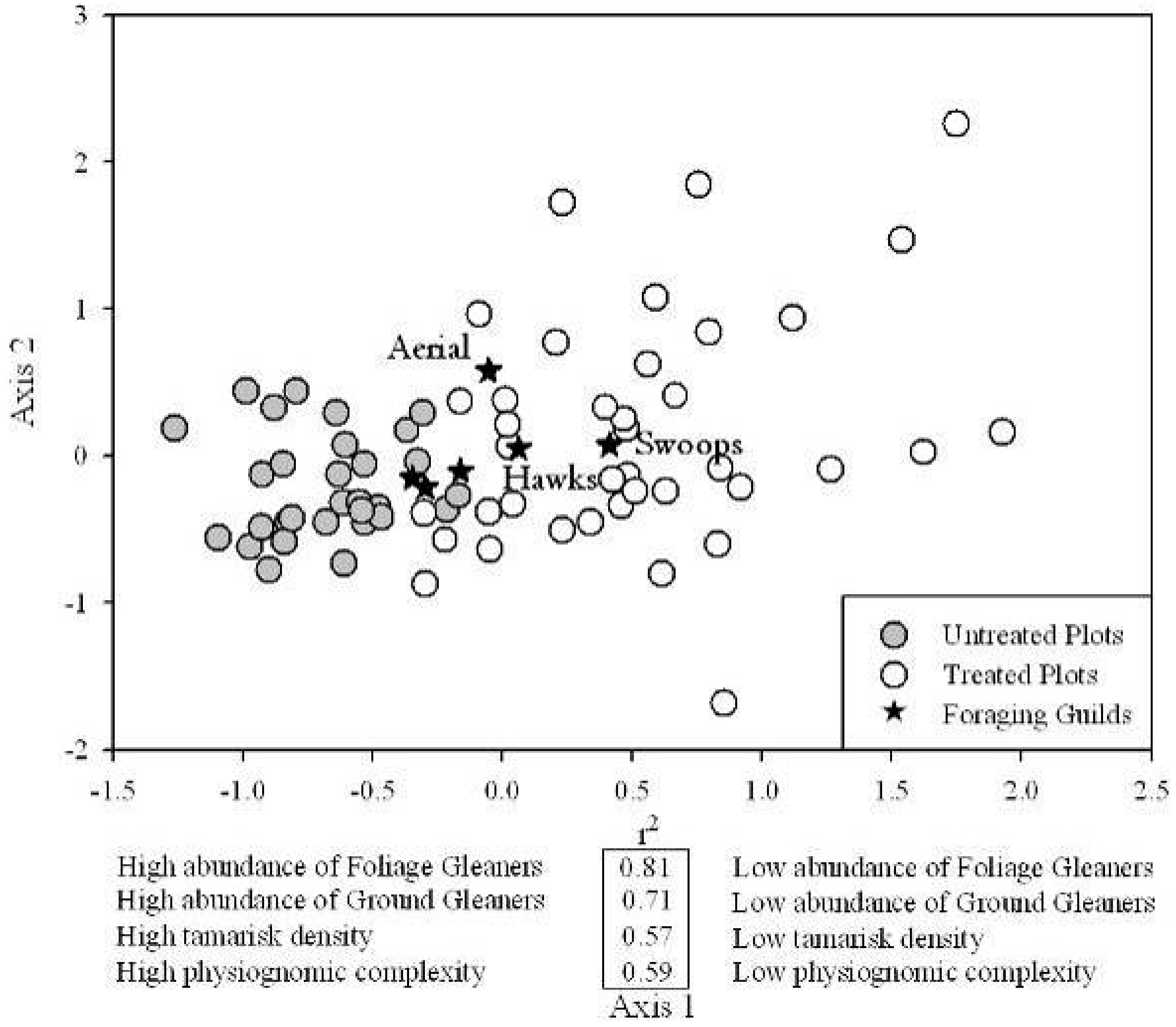


Figure II.3. Non-metric multidimensional scaling ordination for the six avian foraging guilds and six habitat metrics collected at the 69 bird spot mapping plots along the Virgin River, NV. The untreated plots (n=30) had >70% cover of tamarisk while the treated plots (n=39) had tamarisk mechanically removed 2-4 years prior to our surveys. The variables with a coefficient of determination (r^2) of >0.5 are listed on each axis. The three unlabeled foraging guilds are, from left to right, foliage glean, hover and glean, and ground glean.

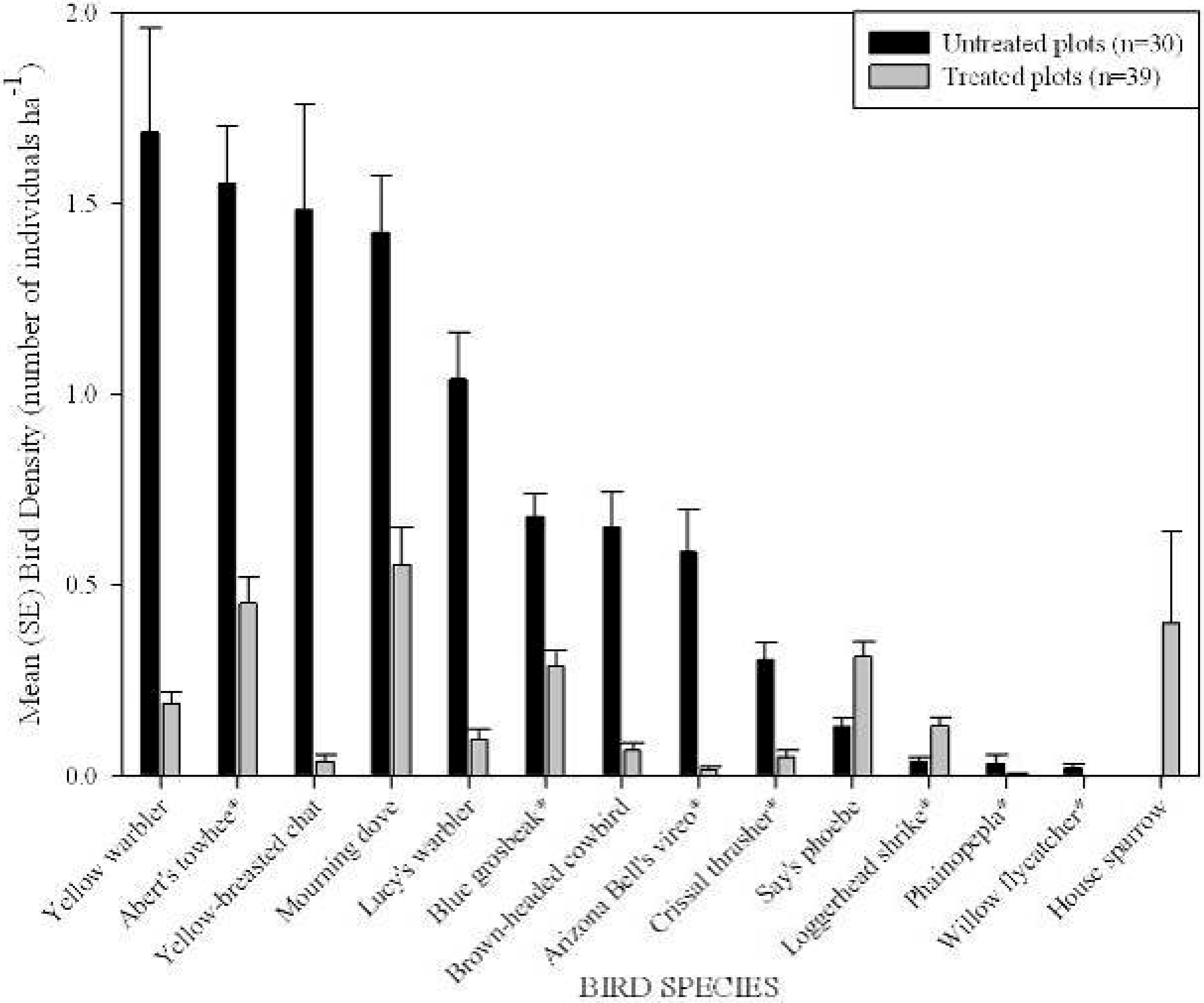


Figure II.4. Mean (plus one SE) bird density as calculated from eight spot map surveys (May-July 2009) along the Virgin River, NV. The untreated plots (n=30) had >70% cover of tamarisk while the treated plots (n=39) had tamarisk mechanically removed 2-4 years prior to our surveys. The species with an asterisk marks bird species that have an official special conservation status in or near the study area.

Table II.1. Monte Carlo test of significance (randomizations = 5,000) of observed maximum indicator value (*IV*) for comparing densities of seven Clark County "covered" bird species in untreated and treated plots along the Virgin River, NV. The untreated (n=30) plots had >70% cover of tamarisk while the treated plots (n=39) had tamarisk mechanically removed 2-4 years prior to our bird surveys (May – July 2009). The *p*-value indicates the proportion of randomized trials with indicator value ≥ the observed *IV* (McCune and Grace (2002).

Bird Species	Observed Indicator Value (*IV*)[a]	IV from randomized groups		
		Mean	Standard Deviation	*p*-value[b]
Abert's towhee	77.5	48.4	3.88	0.0002
Arizona Bell's vireo	64.9	23.0	4.59	0.0002
Blue grosbeak	70.2	47.4	3.70	0.0002
Crissal thrasher	68.9	29.6	4.53	0.0002
Loggerhead shrike	47.9	27.9	4.46	0.0018
Lucy's warbler	91.7	36.4	4.92	0.0002
Phainopepla	8.9	6.1	2.61	0.2678
Southwestern willow flycatcher[c]	13.3	6.1	2.50	0.0280
Yellow-breasted chat	84.6	28.9	5.28	0.0002
Yellow warbler	89.9	46.0	5.35	0.0002

[a] Indicator values range from 0 (no indication) to 100 (perfect indication) with perfect indication meaning that presence of a species points to a particular group without error.

[b] *p* = (1 + number of runs equal to or greater than the observed IV)/ (1 + number of randomized runs)

[c] positively identified as *E. t. extimus* based on multiple sightings

Table II.2. Mean and standard error (SE) of bird densities (individuals ha^{-1}) for each species recorded during eight spot mapping surveys along the Virgin River, NV (April – July 2009).

		Untreated		*Treated*		*Foraging*
Bird Species	*Alpha Code*	*Mean*	*SE*	*Mean*	*SE*	*Guild*[1]
Abert's Towhee	ABTO	1.55	0.15	0.45	0.07	Ground Glean
American Kestrel	AMKE	0.01	0.01	0.04	0.07	Swoops
Anna's Hummingbird	ANHU	0.02	0.01	0.01	0.01	Hover & Glean
Ash-throated Flycatcher	ATFL	0.28	0.05	0.10	0.02	Hover & Glean
Brown-crested Flycatcher	BCFL	0.04	0.03	0.00	0.00	Hover & Glean
Black-chinned Hummingbird	BCHU	0.04	0.03	0.12	0.05	Hover & Glean
Arizona Bell's Vireo	BEVI	0.57	0.11	0.02	0.01	Foliage Glean
Bewick's Wren	BEWR	0.92	0.17	0.03	0.01	Ground Glean
Blue-gray Gnatcatcher	BGGN	0.25	0.05	0.05	0.02	Foliage Glean
Brown-headed Cowbird	BHCO	0.65	0.09	0.07	0.02	Ground Glean
Black-headed Grosbeak	BHGR	0.03	0.01	0.01	0.01	Foliage Glean
Blue Grosbeak	BLGR	0.68	0.06	0.29	0.04	Ground Glean
Black Phoebe	BLPH	0.01	0.01	0.004	0.004	Hawks
Brewer's Sparrow	BRSP	0.12	0.04	0.17	0.06	Ground Glean
Black-tailed Gnatcatcher	BTGN	0.50	0.09	0.11	0.06	Foliage Glean
Black-throated Sparrow	BTSP	0.01	0.01	0.004	0.004	Ground Glean
Black-throated Gray Warbler	BTYW	0.02	0.01	0.02	0.02	Foliage Glean
Bullock's Oriole	BUOR	0.04	0.02	0.02	0.01	Foliage Glean
Chipping Sparrow	CHSP	0.07	0.05	0.02	0.01	Ground Glean
Cooper's Hawk	COHA	0.01	0.010	0.00	0.00	Aerial Pursuit
Costa's Hummingbird	COHU	0.02	0.01	0.01	0.01	Hover & Glean
Common Nighthawk	CONI	0.01	0.01	0.00	0.00	Aerial Forage
Common Raven	CORA	0.07	0.02	0.03	0.02	Ground Glean
Common Yellowthroat	COYE	0.54	0.18	0.05	0.02	Foliage Glean
Crissal Thrasher	CRTH	0.30	0.04	0.05	0.02	Ground Glean
Eurasian Collared-Dove	EUCD	0.00	0.00	0.01	0.01	Ground Glean
Gambel's Quail	GAQU	1.06	0.15	0.51	0.17	Ground Glean
Gray Flycatcher	GRFL	0.01	0.01	0.00	0.00	Hawks
Greater Roadrunner	GRRO	0.20	0.04	0.03	0.01	Ground Glean
Great-tailed Grackle	GTGR	0.02	0.02	0.04	0.02	Ground Glean
House Finch	HOFI	0.06	0.02	0.11	0.04	Ground Glean
Horned Lark	HOLA	0.01	0.01	0.00	0.00	Ground Glean
Hooded Oriole	HOOR	0.01	0.01	0.02	0.02	Foliage Glean
House Sparrow	HOSP	0.00	0.00	0.40	0.24	Ground Glean
Indigo Bunting	INBU	0.49	0.10	0.05	0.01	Foliage Glean
Killdeer	KILL	0.06	0.02	0.24	0.05	Ground Glean
Lark Sparrow	LASP	0.00	0.00	0.004	0.004	Ground Glean
Lazuli Bunting	LAZB	0.41	0.11	0.15	0.05	Ground Glean
Ladder-backed Woodpecker	LBWO	0.01	0.01	0.00	0.00	Bark Glean
Lesser Goldfinch	LEGO	0.03	0.01	0.05	0.02	Foliage Glean
Lesser Nighthawk	LENI	0.12	0.04	0.11	0.03	Aerial Forage
Loggerhead Shrike	LOSH	0.04	0.01	0.13	0.02	Swoops
Lucy's Warbler	LUWA	1.04	0.12	0.09	0.03	Foliage Glean
Marsh Wren	MAWR	0.00	0.00	0.004	0.004	Ground Glean

MacGillivray's Warbler	MGWA	0.05	0.02	0.004	0.004	Foliage Glean
Mourning Dove	MODO	1.42	0.15	0.55	0.10	Ground Glean
Northern Mockingbird	NOMO	0.08	0.02	0.23	0.05	Ground Glean
Northern Parula	NOPA	0.01	0.01	0.00	0.00	Foliage Glean
Northern Rough-winged Swallow	NRWS	0.03	0.02	0.06	0.03	Aerial Forage
Orange-crowned Warbler	OCWA	0.01	0.01	0.00	0.00	Foliage Glean
Phainopepla	PHAI	0.03	0.02	0.004	0.004	Foliage Glean
Plumbeous Vireo	PLVI	0.02	0.01	0.00	0.00	Foliage Glean
Rose-breasted Grosbeak	RBGR	0.01	0.01	0.004	0.004	Foliage Glean
Ruby-crowned Kinglet	RCKI	0.02	0.01	0.004	0.004	Foliage Glean
Rock Wren	ROWR	0.04	0.02	0.00	0.00	Foliage Glean
Red-winged Blackbird	RWBL	0.09	0.04	0.19	0.06	Ground Glean
Say's Phoebe	SAPH	0.13	0.02	0.31	0.04	Hawks
Sora	SORA	0.01	0.01	0.00	0.00	Ground Glean
Song Sparrow	SOSP	1.14	0.23	0.07	0.02	Ground Glean
Summer Tanager	SUTA	0.01	0.01	0.00	0.00	Foliage Glean
Townsend's Warbler	TOWA	0.03	0.01	0.01	0.01	Foliage Glean
Verdin	VERD	0.37	0.08	0.08	0.03	Foliage Glean
Vesper Sparrow	VESP	0.00	0.00	0.004	0.004	Ground Glean
Virginia's Warbler	VIWA	0.01	0.01	0.00	0.00	Ground Glean
Warbling Vireo	WAVI	0.14	0.04	0.01	0.01	Foliage Glean
White-crowned Sparrow	WCSP	0.01	0.01	0.01	0.01	Ground Glean
Western Kingbird	WEKI	0.05	0.02	0.12	0.03	Hawks
Western Meadowlark	WEME	0.03	0.01	0.01	0.01	Ground Glean
Western Scrub-Jay	WESJ	0.01	0.01	0.00	0.00	Ground Glean
Western Tanager	WETA	0.06	0.02	0.02	0.01	Foliage Glean
Western Wood-Pewee	WEWP	0.06	0.02	0.01	0.01	Hawks
Willow Flycatcher	WIFL	0.02	0.01	0.00	0.00	Hawks
Wilson's Warbler	WIWA	0.22	0.04	0.02	0.01	Foliage Glean
Wood Thrush	WOTH	0.01	0.01	0.00	0.00	Ground Glean
White-winged Dove	WWDO	0.03	0.02	0.00	0.00	Ground Glean
Yellow-breasted Chat	YBCH	1.48	0.28	0.04	0.02	Foliage Glean
Yellow-headed Blackbird	YHBL	0.00	0.00	0.02	0.02	Ground Glean
Yellow-rumped Warbler	YRWA	0.02	0.01	0.01	0.01	Foliage Glean
Yellow Warbler	YWAR	1.69	0.28	0.19	0.03	Foliage Glean

[1] Foraging guilds as described in Ehrlich et al. 1998.

Table II.3. Presence of Covered and Special Status avian species in Virgin River ecosystem, based on Effectiveness Monitoring studies (Section II – Control vs. Tamarisk Removal areas) and Tamarisk BioControl monitoring studies (Appendix I – Associations with vegetation types, including Mixed vs. monocultural Tamarisk areas).

			Mean density		**# of records**			
			Effectiveness Monit		**Biocontrol/Vegetation Study**			
Common Name	**Scientific Name**	**Status**	**Control**	**Removal**	**Edge**	**Successional**	**Mixed**	**Tamarisk**
American Peregrine Falcon	*Falco peregrinus anatum*	Covered				1		
Yellow-billed Cuckoo	*Coccyzus americanus*	Covered				0	3	
Vermillion Flycatcher	*Pyrocephalus rubinus*	Covered				1		
Willow Flycatcher	*Empidonax traillii extimus*	Covered[a]	0.02				3	
Phainopepla	*Phainopepla nitens*	Covered	0.03	0.004	8	1	4	2
Summer Tanager	*Piranga rubra*	Covered	0.01		2	6	6	4
Blue Grosbeak	*Guiraca caerulea*	Covered	0.68	0.29	40	46	32	36
Arizona Bell's Vireo	*Vireo bellii arizonae*	Covered	0.57	0.02	27	38	50	26
Western Burrowing Owl	*Athene cunicularia hypugea*	Evaluation						
Bendire's Thrasher	*Toxostoma bendirei*	Evaluation						
LeConte's Thrasher	*Toxostoma lecontei*	Evaluation						
Gray Vireo	*Vireo vicinior*	Evaluation						
Loggerhead Shrike	*Lanius ludovicianus*	Evaluation	0.04	0.13		2	2	1
Crissal Thrasher	*Toxostoma dorsale*	Evaluation	0.3	0.05	10	9	9	9
Western Bluebird	*Sialia mexicana*	Evaluation						
Green-backed Heron	*Butorides striatus*	Watch List						
Western Least Bittern	*Ixobrychus exilis hesperis*	Watch List						
White-faced Ibis	*Plegadis chihi*	Watch List					1	
Yuma Clapper Rail	*Rallus longirostrus yumanensis*	Watch List						
Northern Goshawk	*Accipiter gentilis*	Watch List						
Ferruginous Hawk	*Buteo regalis*	Watch List						
Golden Eagle	*Aquila chrysaetos*	Watch List						
Bald Eagle	*Haliaeetus leucocephalus*	Watch List						
Flammulated Owl	*Otus flammeolus*	Watch List						
Northern Saw-whet Owl	*Aegolius acadius*	Watch List						
Northern Pygmy-owl	*Glaucidium gnoma*	Watch List						
Western Screech-owl	*Otus kennicotti*	Watch List						
Cactus Wren	*Campylorhynchus brunneicapillus*	Watch List						
Canyon Wren	*Catharpes mexicanus*	Watch List			1	1		
Scott's Oriole	*Icterus parisorum*	Watch List						

[a] identified by qualified ornithologist based on multiple sightings

Table II.4. Field notes regarding Covered and Special Status avian species detected in Virgin River studies (follows from Table 3).

Common Name	**Scientific Name**	**Status**	**Notes on off-survey detections/Virgin status**
American Peregrine Falcon	*Falco peregrinus anatum*	Covered	
Yellow-billed Cuckoo	*Coccyzus americanus*	Covered	Detected on surveys at Beaver Dam Wash, AZ. Off-survey, individuals were also detected in Mormon Mesa on two occasions and one possible nest was discovered at Mormon Mesa, though never observed to be active.
Vermillion Flycatcher	*Pyrocephalus rubinus*	Covered	Single individual detected on surveys at Mormon Mesa. Detected off-survey at Thomas Dudley Leavitt Park in Bunkerville, NV, where one male and two females were detected. One successful nest monitored in 2010, two nests found in 2011.
Willow Flycatcher	*Empidonax traillii extimus*	Covered[a]	Southwestern Willow Flycatchers were detected on survey at Beaver Dam Wash, AZ (at two stations), and once on Transect #5 (downstream from Scenic Rd. Bridge). No nests were found or monitored.
Phainopepla	*Phainopepla nitens*	Covered	More abundant earlier in the season (March-April) than during the point count survey period (15 May-30 June). However detections during survey period range from 31 May-22 June, indicating that at least some individuals likely stay on the Virgin River throughout the summer (though many likely migrate to alternative breeding grounds by late April). Off-survey, Phainopeplla were generally found to be most reliably detected among matuure Honey Mesquite stands with ample Mistletoe. We did not locate or monitor any nests for this species.
Summer Tanager	*Piranga rubra*	Covered	Summer Tanagers were regularly detected in low abundance on surveys. They were present on 10 of 20 transects, but only at 15 of 120 point count stations. We did not locate or monitor any nests for this species.
Blue Grosbeak	*Guiraca caerulea*	Covered	Common to abundant in most riparian habitat types on-survey. This species did not seem tied to any particular vegetation type or habitat characteristics besides "edge" type environments. A single nest was found by a point counter while traversing transect 18, but the nest was not monitored through completion.
Arizona Bell's Vireo	*Vireo bellii arizonae*	Covered	Most abundant in habitats with at least some native component (including edge, successional and mixed habitats). Fairly common breeder in the riparian zone and we found and monitored 19 nests through completion, 17 of which were in native/tamarisk mixed nest-searching plots and 2 of which were in monotypic tamarisk.
Loggerhead Shrike	*Lanius ludovicianus*	Evaluation	
Crissal Thrasher	*Toxostoma dorsale*	Evaluation	
White-faced Ibis	*Plegadis chihi*	Watch List	
Canyon Wren	*Catharpes mexicanus*	Watch List	

[a] identified by qualified ornithologists based on multiple sightings

OBJECTIVES 1-3: TAMARISK AND FIRE

OBJECTIVE 1 (partial): *Evaluate relationships between tamarisk, wildfire and impacts to native vegetation and potential for recovery.*

OBJECTIVE 2: *Compare vegetation structure and composition, and tamarisk flammability in relation to foliage condition, in response to experimental herbicide treatments to simulate anticipated defoliation effects of tamarisk biocontrol.*

OBJECTIVE 3. *Compare mortality of tamarisk following fire with and without previous herbicide treatments.*

SECTION III: Wildfire in Tamarisk-invaded Desert Riparian Areas

This Section is comprised of three elements, each fulfilling a different Objective for the Project but fully integrated with each other and not easily treated as independent units. The first (III.1) addresses one part of Objective 1 in the next sub-section, following from the prior two Sections that also address Objective 1. The other two sub-sections (III.2, III.3) are based on Project Objectives 2 and 3 in subsequent portions of this volume.

Background

Significant ecosystem changes resulting from the replacement of native vegetation by dense tamarisk (*Tamarix* spp.) monocultures, and the subsequent reduction of riparian diversity across the American Southwest, may be further impacted by a corresponding increase in riparian fire frequency with tamarisk density (Busch 1995, Gaskin and Schaal 2002). The increase in riparian wildfire observed with tamarisk invasion may represent a novel disturbance, as fire is generally less frequent and severe in riparian areas in comparison to adjacent upland areas due to the high humidity levels, high moisture content of the component vegetation, and lowland location of vegetation where steep banks inhibit the spread of fire into the riparian zone (Agee 1988, Dwire and Kauffman 2003).

Relatively infrequent fire in riparian systems may allow development of biodiversity and maintain ecosystem function by creating refugia for fire sensitive species, and by providing a buffer between higher intensity fires in the upland vs. lowland in-stream environments (Naiman et al. 1993). Natural fires can occur in native riparian systems under conditions of severe drying weather and are thought to be driven by high fuel loads from flood-accumulated debris, but little is known about fire in riparian systems as a whole (Busch 1995, Gregory et al. 2003, Pettit and Naiman 2007). Desert river systems invaded by tamarisk monocultures have been converted from “barriers” into “pathways” for the ignition and spread of fire (Lambert et al. 2011), as tamarisk foliage is more flammable than native foliage (Drus and Paddock, unpub. data) and in dense stands present greater standing biomass and accumulated plant debris than do native stands (Dobyns 1981, Swetnam 1988, Brock 1994, Busch and Smith 1995, Smith et al. 1998, Dudley et al. 2000).

Increases in flammability and fire frequency in riparian zones may threaten native diversity, as riparian species may be unable to adapt to such rapid changes in disturbance regimes, and native species recover more slowly than tamarisk (Heywood 1989, Humphries et al. 1991, Ellis 2001). Fire incidence and tamarisk invasion appear to represent a positive feedback situation in which fire frequency and extent increase with tamarisk density thereby establishing a self-perpetuating, invasive plant/fire regime (Brooks et al. 2004) that can maintain hazardous fuel conditions indefinitely. In association with altered hydrologic regimes, population increases, and forecasted climatic changes, fire may represent a permanent disturbance in this system.

The introduction of tamarisk leaf beetles (*Diorhabda carinulata*) for biological control of tamarisk may be altering the relationship between invasive vegetation and wildfire. Over short temporal scales, herbivory-induced foliar desiccation marginally increases tamarisk flammability, but flammability declines over time as *D. carinulata* herbivory reduces tamarisk growth and subsequent production of fine fuels responsible for ignitions (G. Drus et al., in press). In the absence of herbivory, post-fire tamarisk recovery can range from 50% to 100% depending on the timing and severity of the fire (Ellis 2001, G. Drus, unpub. data). With the introduction of a major herbivore there is potential to break the tamarisk fire cycle and shift towards a native riparian assemblage, as herbivory and fire interact synergistically to cause greater tamarisk mortality together than separately (G. Drus, unpub. data). The threat to native riparian diversity posed by alteration of fire regimes by tamarisk invasion may be mitigated by this new management tool, but data are needed to substantiate the hypothesis that herbivory will yield fundamental change in this relationship.

Following is a series of experimental trials to examine relationships between tamarisk and fire, including consideration of the use of prescription fire for managing tamarisk vegetation.

SECTION III-A Regional Survey of Tamarisk-Fire Relations

Questions Addressed: Does tamarisk invasion promote wildfire in riparian zones, and how is post-burn vegetation composition related to season of fire, pre-burn vegetation composition, and other environmental variables?

Methods - Fire data and site selection

In a 2010 survey of tamarisk-fueled fires in southwestern desert riparian ecosystems (Mojave, Sonoran, Chihuahuan, and Great Basin; Fig. III.1), residual fuel structure, biomass consumption, and understory composition of woody native vs. non-native species in recently burned desert riparian corridors were characterized as a function of burn timing, pre-burn tamarisk density, and unburned fuel structure. Sites were selected using the Monitoring Trends in Fire Severity (MTFS) Database, InciWeb (http://www.inciweb.org/), ESRI (http://www.esri.com/library/whitepapers/pdfs/fire-service-gis-applications.pdf), and data provided by individual government agencies (Bureau of Land Management - BLM, National Park Service - NPS, Bureau of Reclamation - BOR, etc.). To be selected for the survey, pre-burn vegetation composition, type of burn (prescribed, wildfire), time since burn, and season of burn could be documented for a given site.

Fuel Structure and plant community composition Each burn site was separated into different areas based on pre-burn vegetation structure and post-burn severity in an area, paying particular attention to capturing variability in pre-burn tamarisk density. Within the different burn areas, 30 m brush-belt transects were used to describe the post-fire (residual) fuel structure of woody species, where all woody individuals with a base within 2 m on each side of the transect tape were measured. At each burn site, at least 1 unburned 30m transect was conducted to describe unburned native and non-native vegetation, and at least 2 burned 30m transects were conducted to describe burned vegetation. The total transects performed depended on the size and complexity of the fire. The stratified random assignment of transects within different burn areas allowed for a more complete description of the relationships within burn sites, given spatial limitations and potential issues with autocorrelation(van Mantgem and Schwilk 2009).

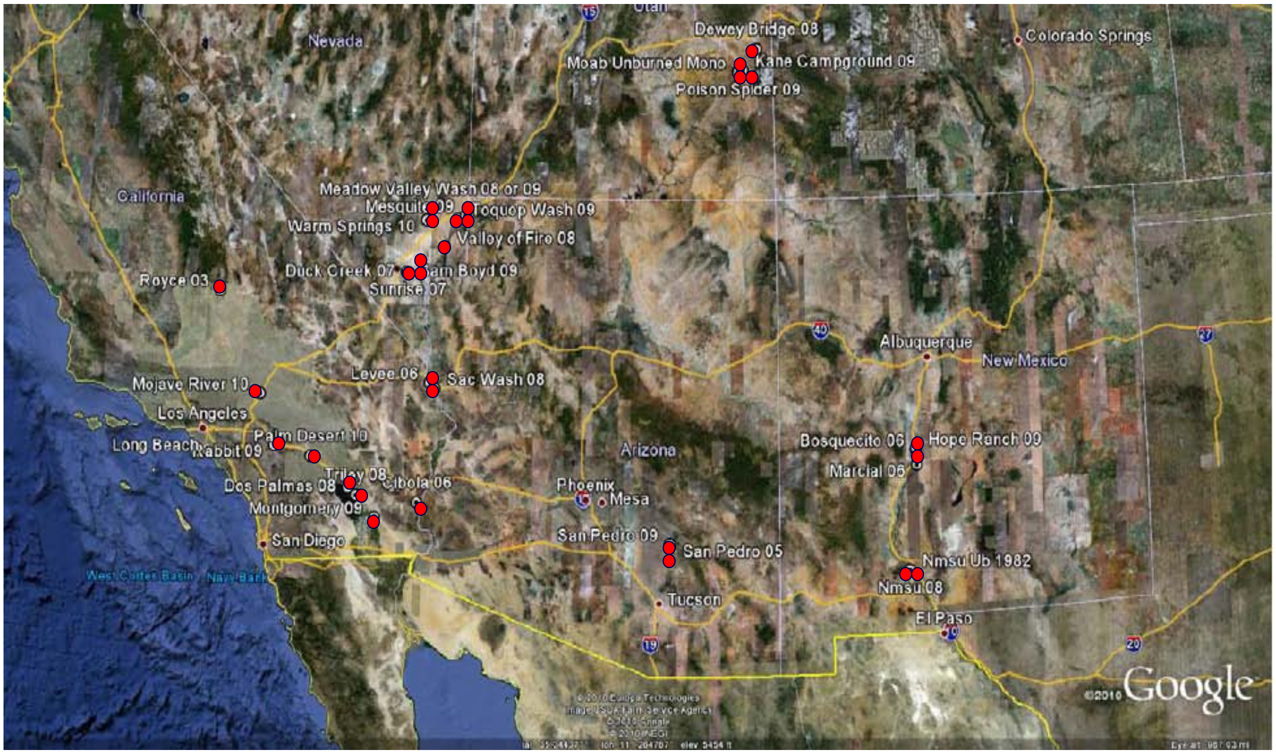
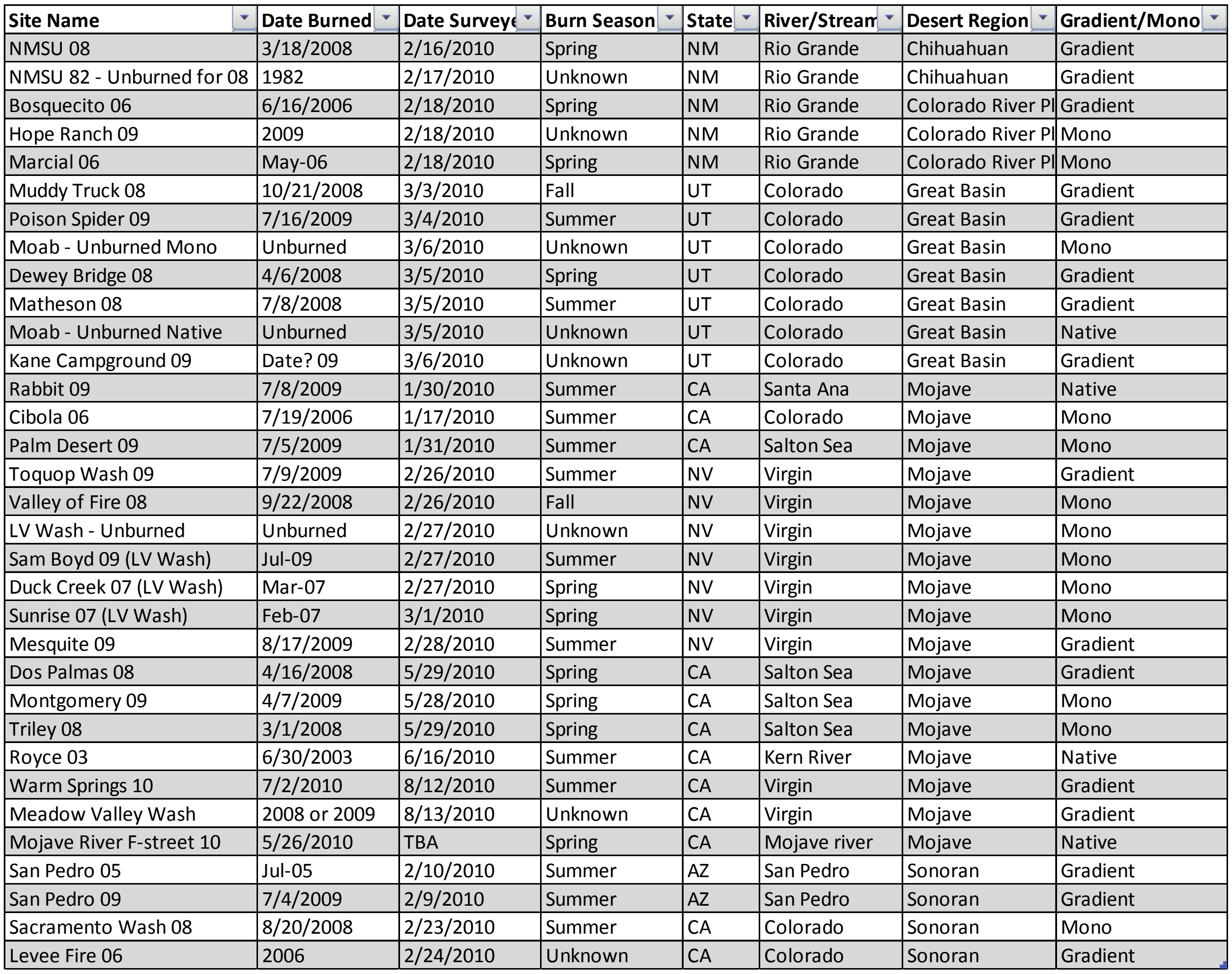


Figure III.1. Fire survey site locations.



Site Name	Date Burned	Date Surveye	Burn Season	State	River/Stream	Desert Region	Gradient/Mono
NMSU 08	3/18/2008	2/16/2010	Spring	NM	Rio Grande	Chihuahuan	Gradient
NMSU 82 - Unburned for 08	1982	2/17/2010	Unknown	NM	Rio Grande	Chihuahuan	Gradient
Bosquecito 06	6/16/2006	2/18/2010	Spring	NM	Rio Grande	Colorado River Pl	Gradient
Hope Ranch 09	2009	2/18/2010	Unknown	NM	Rio Grande	Colorado River Pl	Mono
Marcial 06	May-06	2/18/2010	Spring	NM	Rio Grande	Colorado River Pl	Mono
Muddy Truck 08	10/21/2008	3/3/2010	Fall	UT	Colorado	Great Basin	Gradient
Poison Spider 09	7/16/2009	3/4/2010	Summer	UT	Colorado	Great Basin	Gradient
Moab - Unburned Mono	Unburned	3/6/2010	Unknown	UT	Colorado	Great Basin	Mono
Dewey Bridge 08	4/6/2008	3/5/2010	Spring	UT	Colorado	Great Basin	Gradient
Matheson 08	7/8/2008	3/5/2010	Summer	UT	Colorado	Great Basin	Gradient
Moab - Unburned Native	Unburned	3/5/2010	Unknown	UT	Colorado	Great Basin	Native
Kane Campground 09	Date? 09	3/6/2010	Unknown	UT	Colorado	Great Basin	Gradient
Rabbit 09	7/8/2009	1/30/2010	Summer	CA	Santa Ana	Mojave	Native
Cibola 06	7/19/2006	1/17/2010	Summer	CA	Colorado	Mojave	Mono
Palm Desert 09	7/5/2009	1/31/2010	Summer	CA	Salton Sea	Mojave	Mono
Toquop Wash 09	7/9/2009	2/26/2010	Summer	NV	Virgin	Mojave	Gradient
Valley of Fire 08	9/22/2008	2/26/2010	Fall	NV	Virgin	Mojave	Mono
LV Wash - Unburned	Unburned	2/27/2010	Unknown	NV	Virgin	Mojave	Mono
Sam Boyd 09 (LV Wash)	Jul-09	2/27/2010	Summer	NV	Virgin	Mojave	Mono
Duck Creek 07 (LV Wash)	Mar-07	2/27/2010	Spring	NV	Virgin	Mojave	Mono
Sunrise 07 (LV Wash)	Feb-07	3/1/2010	Spring	NV	Virgin	Mojave	Mono
Mesquite 09	8/17/2009	2/28/2010	Summer	NV	Virgin	Mojave	Gradient
Dos Palmas 08	4/16/2008	5/29/2010	Spring	CA	Salton Sea	Mojave	Gradient
Montgomery 09	4/7/2009	5/28/2010	Spring	CA	Salton Sea	Mojave	Mono
Triley 08	3/1/2008	5/29/2010	Spring	CA	Salton Sea	Mojave	Mono
Royce 03	6/30/2003	6/16/2010	Summer	CA	Kern River	Mojave	Native
Warm Springs 10	7/2/2010	8/12/2010	Summer	CA	Virgin	Mojave	Gradient
Meadow Valley Wash	2008 or 2009	8/13/2010	Unknown	CA	Virgin	Mojave	Gradient
Mojave River F-street 10	5/26/2010	TBA	Spring	CA	Mojave river	Mojave	Native
San Pedro 05	Jul-05	2/10/2010	Summer	AZ	San Pedro	Sonoran	Gradient
San Pedro 09	7/4/2009	2/9/2010	Summer	AZ	San Pedro	Sonoran	Gradient
Sacramento Wash 08	8/20/2008	2/23/2010	Summer	CA	Colorado	Sonoran	Mono
Levee Fire 06	2006	2/24/2010	Unknown	CA	Colorado	Sonoran	Gradient

Table III.1: Fire survey sites and descriptions.

Species identification (or to lowest taxonomic level possible), maximum height and canopy width (in 2 perpendicular planes) for live and dead material were measured with tree poles and metal tapes for all woody individuals along each transect. As tamarisk and some willow species have multiple stems, what constituted and individual was determined subjectively. Five stem diameters were measured at the ground surface and at breast height. Presence or absence of timelag fuel classes were recorded, where the diameters of 1hr, 10hr, 100hr and 100hr fuels are $\leq$ 0.6, 0.6-2.5, 2.5-7.6, and >7.6 cm respectively (Pyne et al. 1996). Percent live and dead canopy volume composed of 1hr fuels was estimated to indicate fine fuel consumption and recovery. Understory composition was measured using three 1x1 m quadrats randomly placed along each transect and chosen using a random numbers table. For each quadrat, height and percent cover of each species was recorded. Unknowns were photographed in the field and collected in a plant press for subsequent identification. Reference photographs were taken of most transects and quadrats. Data were collected from 33 sites across the southwestern states (Table III.1).

*Statistical analysis*_All statistical analyses were performed with JMP version 8 (SAS Institute 2008) using a critical value of 0.05. Standard error bars were included in all figures using non-transformed data for the purposes of visual display (Anderson et al. 2001), while statistical comparisons were made among sample population means (i.e. sites)(SAS 2008). Proportional data were arcsine-square-root transformed to improve normality. The effect of tamarisk density on native fuel consumption (indicated by the proportion of 1hr fuels by the total residual fuel classes) was evaluated using one-factor ANOVA. The recovery of tamarisk vs. native species was analyzed as a function of tamarisk density classes using 2-factor ANOVA. Logistic regression was used to evaluate native mortality as a function of tamarisk density.

Results

Tamarisk invasion is increasing the flammability of southwestern riparian zones, and is displacing native species following fire. As tamarisk density increased, the consumption of native fine fuels increased across survey sites ($F_{2,73}$ = 9.3221, P = 0.0003; Fig. III.2), indicating that tamarisk is more flammable than native species at the stand level. Conversely, native species retain the majority of fine fuels following fire in the absence of or at low-levels of tamarisk density. Further, as tamarisk density increased, native recovery decreased and tamarisk recovery increased ($F_{2,73}$ = 9.3221, P = 0.0003; Fig. III.3), indicating that tamarisk recovers more efficiently from fire than natives, and promotes itself. Willow and cottonwood mortality increased with tamarisk density (Cottonwood: R^2 = 0.07, χ^2 = 78.89, P = 0.0014, DF = 107. Willow: R^2 = 0.26, χ^2 = 31.56, P < 0.001, DF = 97; Fig. III.4), suggesting that tamarisk-fueled fires will replace native species over time as tamarisk invasion continues.

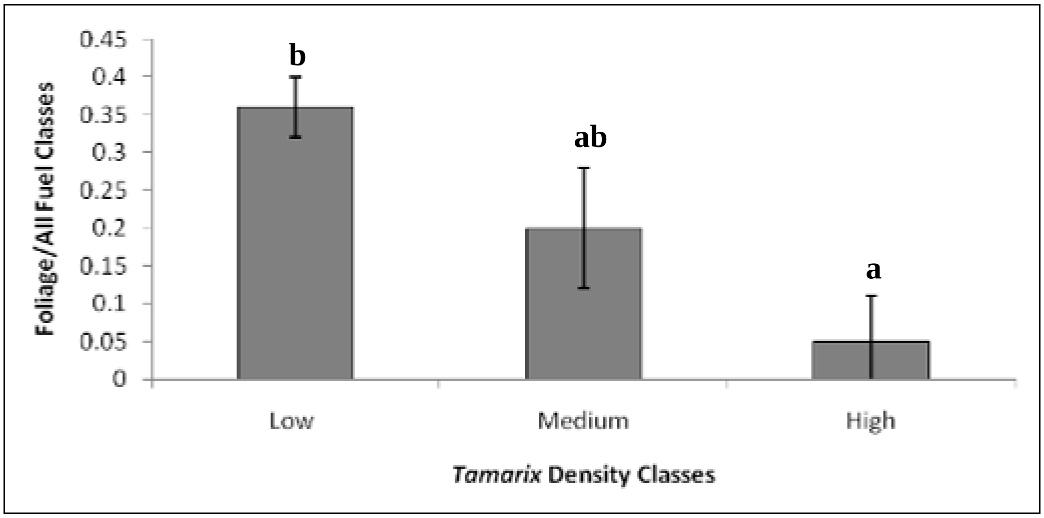


Figure III.2. Fuel consumption represented by the incidence of fine fuel class (foliage) divided by incidence of all fuel classes remaining from a burn. Tamarisk (*Tamarix*) fuel classes are defined as follows: Low = < 10% cover; Medium = 20-20% cover; High = > 50% cover. Error bars indicate one +/- standard error; letters (a and b) indicate pairs showing significant differences ($p \leq 0.05$) among density classes (matched letters not significantly different).

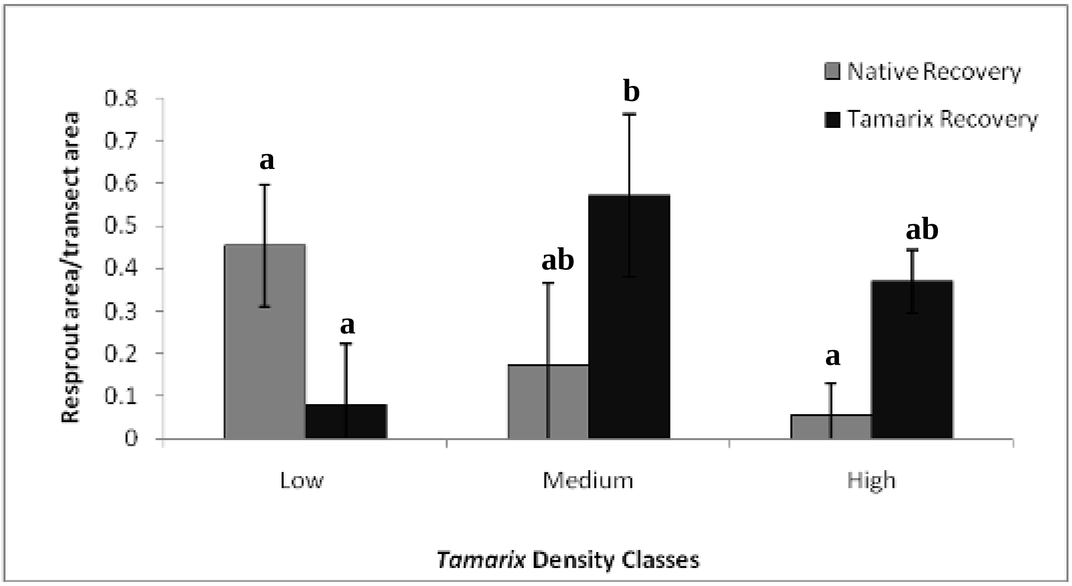


Figure III.3: Recovery is indicated by the area of resprouts divided by the transect area. Tamarisk (*Tamarix*) fuel classes are defined as follows: Low = < 0% cover; Medium = 20-20% cover; High = > 50% cover. Error bars indicate one +/- standard error; letters (a and b) indicate significant differences ($p \leq 0.05$) among density classes within species groupings.

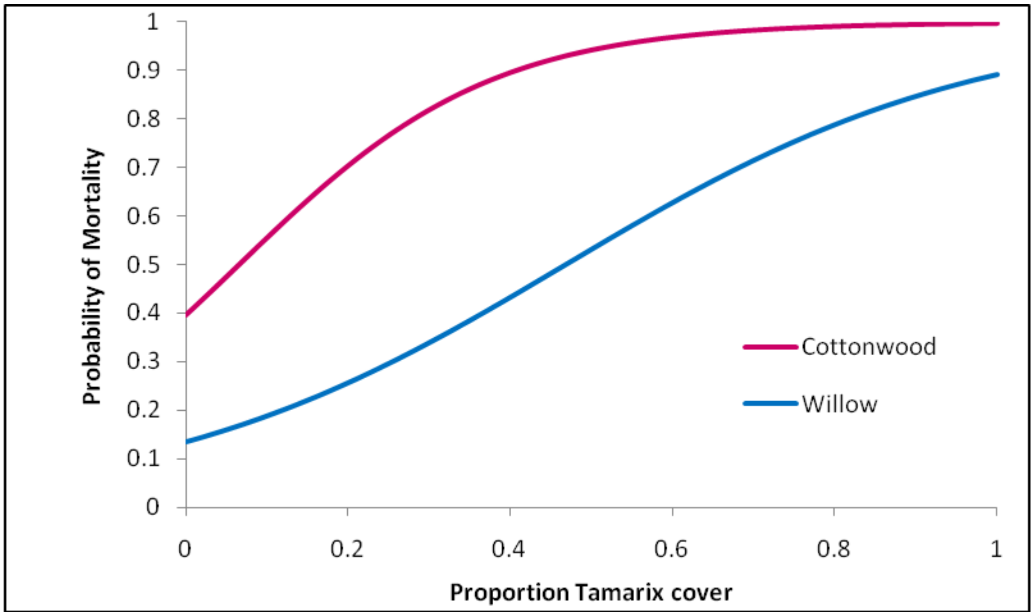


Figure III.4: Probability of mortality of cottonwood and willow as a function of Tamarisk (*Tamarix*) density derived via polynomial regression.

SECTION III. TAMARISK AND FIRE
Sub-Section III.B Tamarisk Condition and Flammability

Questions addressed: What foliage conditions enhance the flammability of tamarisk, and what mechanisms produce these foliage conditions (ie. reduction in fuel moisture due to biocontrol, herbicide application, drought, etc.).

Materials and Methods

Study Sites *Diorhabda carinulata* herbivory level (high vs. low) was evaluated at a first site in northern Nevada, and comparisons were made between fires during August and October of 2006. The site was located in the lower Humboldt River basin (HB) in central Nevada (40.07˚N, 118.5˚W) where 15,000 hectares of land are dominated by tamarisk vegetation (*Tamarix* spp.; Sengupta et al. *2*004). Vegetation included saltgrass (*Distichlis spicata*), greasewood (*Sarcobatus vermiculatus*), and saltbush (*Atriplex spp.*), as well as non-native forbs (Russian knapweed – *Acroptilon repens,* tall whitetop – *Lepidium latifolium,* halogeton – *Halogeton glomeratus,* and fivehook bassia – *Bassia hyssopifolia*). Intermittent grazing, periodic flooding and wildfire were common in this area. Tamarisk at the study site was subjected to three full seasons of defoliation by *Diorhabda* before experimental fires began. Extensive dieback (10% mortality, and 60-80% desiccation resulting from herbivory by *D. carinulata*) had occurred and the amount of regrowth was variable among trees.

Herbicide treatment (herbicide vs. no herbicide) was evaluated at a second site in southern Nevada with a burn conducted during September 2008. The site was located in Valley of Fire Wash (VOF; 36.41˚N, 114.39˚W), where the wash is dominated by *Tamarix ramosissima*). Vegetation included arroweed (*Pluchea sericea*), catclaw acacia (*Acacia greggii*) and remnant populations of screwbean mesquite (*Prosopis pubescens*) and Gooding's willow (*Salix goodingii*) at the edge of the tamarisk monoculture. Common reed (*Phragmites australis*), and other desert annuals were found in lower densities. Intermittent grazing, periodic flooding and wildfire were common disturbances in this area.

Experimental Treatments At HB, we evaluated the effect of herbivory (low to high) on fire behavior with prescribed fires during two seasons (August and October). Each treatment (August, October, unburned) was replicated by three 0.5ha plots (4.5 ha total). Plots were separated by bulldozed firebreaks, ~6 m in width or roughly twice the height of the vegetation, which allowed multiple burns to be conducted within each burn season. At VOF, we evaluated the effect of herbivory on fire behavior with a single prescribed fire (September), using herbicide treatments to simulate herbivory. Simulated herbivory and control treatments were applied across six plots, where each treatment was replicated with three 30 x 30 m plots, covering 0.54 ha within an overall fire area of 8.1 ha. Certified National Park Service personnel applied 10% glyphosate to the foliage to simulate herbivory desiccation, followed by a controlled burn 4 weeks later. For the purposes of this study, herbicide was applied only to desiccate the foliage over the short-term to simulate the level of desiccation caused by biocontrol herbivory at other sites.

Spatial autocorrelation and pseudo-replication are often unavoidable issues in studies conducted at large spatial scales (Legendre 1993), but for fire studies in particular because sub-sampling is commonly done within large burn areas. Although these effects should be examined when possible, they have been shown to be negligible when compared to the overall fire effects, which vary with vegetation structure and weather within a single burn (van Mantgem and Schwilk

2009). Given the homogeneous nature of the tamarisk monoculture at both sites, we used a stratified random approach to assign treatments to individual plots, and to select individual trees within each plot. This approach allowed effective comparisons to be made using parametric models despite spatial limitations

Fuel Characteristics Point transects, quadrats and circular plots were used to describe vegetation characteristics within experimental plots before and after burn treatments. Point transects consisted of a set transect length (HB 50 m, VOF 10 m) with intercepts at regular intervals (HB 0.5 m, VOF 1 m) where the height and species of vegetation within 10 cm of the intercept pole were recorded. Transects (HB 2 per plot, VOF 1 per plot) measured vegetation before and 1 year following the burn. At VOF, 18 additional transects were run perpendicular to the stream channel to capture the variability of vegetation at the site. Quadrats (HB 0.25 m^2 , VOF 1 m^2) were placed at regular intervals (HB 10m, VOF 5 m) to measure the abundance, average height, and percent cover of each plant species, and to measure litter depth. Circular plots with a 5 m radius were placed within each treatment plot (HB 4 per plot, VOF 2 per plot) to determine tamarisk density for biomass estimation. For each tree within the circular plots, maximum height and canopy diameter were measured with tapes and calibrated PVC poles, and percent foliage (green foliage volume per total canopy volume) was visually estimated to the nearest 5%.

Destructive sampling was used to estimate fuel loading (dry biomass per unit area), and fuel moisture ([mass of wet fuels – mass of dry fuels]/ mass of wet fuels) of fine fuels (fast-drying fuels less than 0.6 cm in diameter) at the time of the prescribed fires. The biomass of tamarisk plant material per unit area was calculated by harvesting 15 trees within 20 m of the fire plots, and by using density data from the 5 m radius circular plots. The trees were chosen to represent size classes from the smallest to largest trees in the burn plots. Each tree was separated into fuel diameter classes named according to the timelag principle where small diameter fuels change more rapidly in response to weather changes than larger diameter fuels; timelag classes used included 1 hr (< 0.625 cm), 10 hr (0.625 – 2.5 cm), 100 hr (2.5 – 7.6 cm) and 1000 hr (> 7.6 cm) as defined by (Anderson 1982). Timelag classes were bagged and weighed with a handheld spring scale for each tree. To calculate the biomass per unit area for each 5 m radius circular plot, physical parameters (height, canopy width, % green foliage) were regressed against biomass. Canopy width was the strongest predictor of biomass by non-linear regression at HB, as the trees were round in shape, and canopy was incorporated into the formula $Y = 1.010X^{2.863}$ as the X variable ($r^2 = 0.96$, $P = 0.03$). At VOF, irregular tree shape and other physical parameters potentially influencing biomass were described using the following index:

$$\frac{\text{Height} * \text{Average Canopy Width} * \text{Average Basal Width} * \text{Proportion Foliage}}{\left(\frac{\text{Height}}{\text{Average Canopy Width}}\right) * \text{Canopy Ratio}}$$

which was then correlated with biomass to produce the formula $Y = -0.0002X^2 + 0.1993X + 8.2$ ($r^2 = 0.9384$, $P = 0.009$), with the shape index as the X variable. Width at the ground level (basal width) was measured for stems comprising each individual tree. Canopy ratio was the larger canopy width divided by the smaller canopy width, and indicated the general shape of the trees (ratios approaching 1 indicated round trees, while smaller ratios indicated trees elongated on 1 axis, and truncated on the other). Foliage samples were taken from burn plots preceding the prescribed fire to obtain fuel moisture estimates, and were oven dried at 60˚C until dry (4 to 7 days) to determine water content.

Trees within treatment plots were marked at the base with numbered steel washers, driven into the soil with 30 cm steel nails for monitoring (HB 30 per plot, VOF 21 per plot). We chose tamarisk trees that were clearly individuals with multiple stems branching from a single base. For each marked tree, total height, average canopy diameter, height and species of understory plants and litter were measured, and percent green vs. brown (desiccated) foliage and percent cover of understory species were visually estimated. At VOF, simulated herbivory resulted in desiccation of 46.8% of the canopy foliage, while the control indicated no desiccation. Because the entire region surrounding the HB site was colonized by the *D. carinulata* prior to the experiment, there was no unaffected control treatment with no history of biocontrol herbivory. Instead, a selection of trees with varying herbivory levels was utilized; of the 30 trees per plot, 15 low herbivory (mean 42% desiccated foliage) and 15 high herbivory (mean 85% desiccated foliage) trees were selected to investigate the effect of varied beetle desiccation on fire behavior.

Fuel Manipulation Litter was manipulated on selected trees to determine the influence of litter on fire intensity, strictly defined as a measure of the rate of heat released by a fire, including both radiant and convectional heat (Tangren 1976). For the purposes of this study, the term 'fire intensity' refers to a Fire Intensity Index (FII) expressed in degree-minutes above 70˚C, the temperature at which most plant tissue dies (Lepeschkin 1938), to infer damage to plant tissues. A subset of monitored trees per fire plot (HB 10, VOF 4) was assigned to litter removal, litter addition (HB 7.5 cm, VOF 20cm) treatments, unmanipulated control litter manipulations. Litter was collected from the understory of tamarisk adjacent to the burn plots, and from similar infestations in the study areas. Litter depths of 7.5 cm and 20 cm were chosen for HB and VOF, to represent maximum litter depths measured at the respective sites. Trees were selected in a stratified random design to investigate the interactive effects of herbivory and litter manipulation; 5 trees were selected to represent low beetle impact (Avg 42% desiccated foliage), and 5 trees were selected to represent high beetle impact (mean 85% desiccated foliage) of the 10 trees selected for litter manipulation in each plot at the HB site.

Prescribed fire treatments Firebreaks were established at HB using a bulldozer to allow for unburned control plots and multiple prescribed fires which were conducted between 1300 and 1700 hours in August (21, 22, and 24) and October (27, 28, and 29) 2006. At VOF a single prescription burn was conducted on September 26, 2008 between 1100 and 1700 hours. Drip torches containing a 1:3 gasoline to diesel mixture were used to ignite backing fires (fires spreading against the wind), flanking fires (fires moving perpendicular to the wind) and heading fires (fires moving with the wind), and maintained by igniting in a ring around the plots at HB, and by flare guns and fuses at VOF. Ambient temperature, wind speeds, and relative humidity were recorded hourly at each site; at HB data were obtained from the United States Historical Climatology Network Station at Derby Field Airport located 1.5km west of the study site, and at VOF data were collected with a Kestrel 2000 handheld weather meter.

Type K Nickel-Chromium/Nickel-Aluminum thermocouples and Campbell Scientific CR10X dataloggers recorded temperature every 30 seconds during all prescribed fires to obtain fire temperature over time. To compare fire behavior among different vegetation layers, thermocouples were placed at different levels: aboveground in the canopy (HB 1 m, VOF 2 m) within the litter (HB only), at the litter-soil interface (near the root crown) beneath each monitored tree (HB 30 per plot, VOF 9 plot), and 2 cm below the surface of the mineral soil (HB 2 per plot) to examine heat penetration. Significant interactions between thermocouple placement and litter manipulation were expected because the thermocouples were placed at the site of litter manipulations. Thermocouples were excluded from litter manipulations at VOF. Because there were a limited number of thermocouples, they were placed more judiciously among the plots at VOF. Rate of fire spread in m min^{-1} at HB was calculated using the ignition time of each monitored tree and dividing by the distance between trees using a map generated by ArcGIS version 9.3 9 (HB), and visually estimated at the time of the fire (VOF). Flame lengths were visually estimated during the fires and from video recordings using fire poles and other reference objects of known scale.

Statistical Analyses All statistical analyses were performed with JMP version 8 (SAS 2008) using a critical value of 0.05. Proportional data were arcsine-square-root transformed, and fire intensity index data (FII) were natural-log transformed to improve normality for parametric analysis. Standard errors were included in all figures for the purposes of illustrating sample variability, while statistical comparisons were made among sample population means (i.e. treatment plots).

Fire weather parameters (relative humidity, temperature, and wind speed) were compared between August and October fires at HB using two-factor Analysis of Variance (ANOVA; Zar 2009). One-factor ANOVA was used to compare rate of spread and flame length by fire per foliar treatment, maximum temperature and fire duration by fire per foliar treatment, litter depth by plot, tree height by plot, and tamarisk and understory cover removal by plot. Vegetation removal by fire per foliar treatment (August vs. October, herbicide vs. non herbicide) and census timing (pre vs. post-fire) were analyzed with two-factor ANOVA.

For HB only, three-factor ANOVA was used to compare fire duration data as a function of burn treatment, litter treatment, thermocouple position and full factorial interactions. Analysis of Covariance (ANCOVA) was used to analyze tamarisk removal as a function of pre-fire density. Three-factor ANOVA analyzed natural log transformed fire intensity data or FII as a function of burn treatment, litter treatment, and thermocouple position. One-factor ANOVA analyzed the effect of *Diorhabda* herbivory on FII by herbivory level (mean 42% desiccated foliage) and 15 high herbivory (mean 85% desiccated foliage),and fire season. Tukey-Kramer HSD was used for multiple comparisons of means.

For VOF data only, three-factor ANOVA analyzed arcsine square root transformed foliage moisture by treatment (herbicide vs. no herbicide), sample, plot and full factorial interactions. FII (degree-minutes above 70˚C) and maximum temperature data were analyzed as a function of foliage treatment, thermocouple position, and the interaction between foliage treatment and thermocouple position using two-factor ANOVA.

Foliar Flammability Experiments

Collection site Foliar samples were collected from the Santa Clara River near Piru, CA (34.41˚N, 118.7˚W), a 4th order low-gradient riparian system in the interior Coast Range. This site is semi-arid with moderately high salinity, and based on common understory vegetation very similar to that found at Mojave Desert riparian sites [saltgrass (*Distichlis spicata*), heliotrope (*Heliotropium curassavicum*), quailbrush (*Atriplex lentiformis*), arroweed (*Pluchea sericea*), giant reed (*Arundo donax*), common cattail (*Typha latifolia*), bulrush (*Scirpus* spp.)] it was assumed that source location would have no significant effect on flammability traits. The common tree species included coyote willow (*Salix exigua*), arroyo willow (*Salix lasiolepis*), Fremont cottonwood (*Populus fremontii*), Gooding's willow (*Salix goodingii*) and tamarisk (*Tamarix ramossisima* at this site).

Experimental Treatments Experimental burn trials compared the flammability (ignitability, and combustibility) of desiccated vs. undesiccated tamarisk and native vegetation (*S. exigua*, *S. goodingii*, *P. fremontii*) at the level of individual leaves, and the distal 10 cm of foliage from tree branches. That distal 10 cm sample was to represent the most likely site of ignition when foliage is exposed to high levels of convection from high intensity upland fires moving into the riparian corridor. The volume of leaf or distal foliage samples was determined using water displacement by inserting individual leaves into graduated cylinder. Leaf area of both sample types was calculated by scanning leaves or foliage samples using a Lexmark 2300 scanner$^{©}$, and leaf area was determined using Image 'J'.

The proportion of samples producing flame was determined for six leaves from each species at 2 moisture levels (desiccated vs. undesiccated). Time to ignition was determined from 6 live distal 10 cm foliage samples per species. 'Desiccated' samples were dried 24 hours in a Labline drying oven set to 80˚C to provide different foliage moisture levels, and to determine volumetric foliage moisture by weighing pre- and post-drying.

Ignition method A Fisher Scientific Isotemp Muffle Furnace was set to 650°C and used to ignite plant samples (Montgomery and Cheo 1969; Fig. III.5). Unlike a flame or point ignition source where the ignitability of fuels may be influenced by environmental parameters, the muffle furnace provides an evenly distributed, flameless convective ignition source. Ignition was defined as any combustion of leaf material; glowing (charring) without flame, or production of a flame. Individual leaves and 10 cm foliar samples were placed inside and a stopwatch was used to measure time to smoke and fire (ignitability), total flame time (sustainability), and time to total consumption. Sufficient time was allowed between samples for the furnace to re-establish the set temperature of 650°C. Consumability was measured for individual leaves using the following expression:

Figure III.5. Foliar flammability apparatus composed of alligator clips, stainless steel wire, secured to a 10 cm terra cotta tray. Glowing or charring is shown in a, flaming is shown in b, and consumption is shown in c.

[pre-fire weight – post-fire weight]/ pre-fire weight

Statistical analysis All statistical analyses were performed with JMP version 8 (SAS 2008) using a critical value of 0.05. Standard error bars were included in all figures for the purposes of visual display, while statistical comparisons were made among sample population means (i.e. species). The relative proportion of individual leaf samples producing flame in desiccated vs. undesiccated samples were analyzed using contingency table analysis. One-factor ANOVA was used to evaluate the relationship between time to ignition among species.

Results: Tamarisk Condition and Flammability

At the HB site, pre-burn tamarisk plants ranged from 2 to 4m in height, and the aerial cover averaged 44%, which did not differ between August and October treatment plots ($F_{1,20} = 1.83$, $P = 0.19$; Table III.2). The tamarisk stand was composed of young age-class trees which established in a 1983 flood event (Gail Munk, University of Nevada Coop. Extension, pers. comm.) with foliage throughout the tree and a fairly developed understory. The understory was comprised primarily of non-native plant species, ranging from 0.25 m to 0.75 m in height with an average of 52.8% cover, which did not differ between August and October treatments ($F_{1,20} = 0.052$, $P = 0.82$). Litter in the understory of tamarisk was 3.4 cm deeper than litter out in the open, or in the interstices between trees, but these depths were not different across plots ($F_{5,174} = 1.15$, $P = 0.3338$). However, the southernmost plot had 3.1 cm deeper litter in the interstices than the other 8 plots ($F_{8,81} = 8.63$, $P < 0.0001$). Total fuel load was estimated at 14,500 Kg ha^{-1}, with approximately 30% of the biomass comprised of fine fuels.

Table III.2. Fuel structure by site. Values are means +/- one standard error. Sample size varied by site and vegetation/fuel parameter. * indicates $p \leq 0.05$ among treatments within a site.

Site	*Tamarix* Ht (m)	*Tamarix* Cover (%)	Litter Depth (cm)	Fuel Load (Kg ha^{-1})
Humboldt	2.62 m ± 0.03	44% ± 5.4	4.7 cm ± 0.1	14,500 Kg ha^{-1}
VOF	4.50 m ± 0.18	95% ± 0.08	9.1 cm ± 1.0	212,779 Kg ha^{-1}

At VOF, prior to the fire tamarisk trees ranged from 2-5.5 m in height, and its aerial cover averaged 95%, which did not differ between foliage treatments ($F_{1,47} = 0.0064$, $P = 0.94$). The tamarisk monoculture was composed of larger trees, with the majority of foliage located in the upper canopy and a poorly developed live understory vegetation. Understory cover was comprised of 37.5% (± 0.07 SE) tamarisk debris (woody material and foliage) and 88.8% (± 0.07 SE) tamarisk foliar litter, with *Pluchea sericea* occurring at plot edges, and rarely in the understory of smaller trees found at the fringes of the infestation at wash edges. Understory cover was not different among treatment plots ($F_{1,70} = 0.66$, $P = 0.42$; Table III.2). Litter depth averaged 9.1 cm, and did not differ by herbicide treatment ($F_{1,53} = 1.85$, $P = 0.17$). Total fuel load was estimated to be 212,779 Kg ha^{-1}. Fine fuels comprised 26.8% (± 5.7 SE) of the tree biomass.

At HB, the experimental fires removed 44.8% of the original 44% tamarisk cover (Table III.2; $F_{1,10} = 0.68$, $P = 0.43$), and 27.3% of the original 52.8% understory cover (Fig. III.6; $F_{1,10} = 0.16$, $P = 0.69$), which did not differ between August and October fires (Fig. III.6; $F_{1,20} = 1.2$, $P = 0.29$). Tamarisk biomass consumption increased as a function of pre-fire density during both burn seasons ($F_{1,8} = 13.37$, $P = 0.0017$). At VOF, the experimental fire removed 66% of the original 95% tamarisk material, and did not differ by herbicide treatment (Fig. 3; $F_{1,47} = 0.0064$, $P = 0.94$).

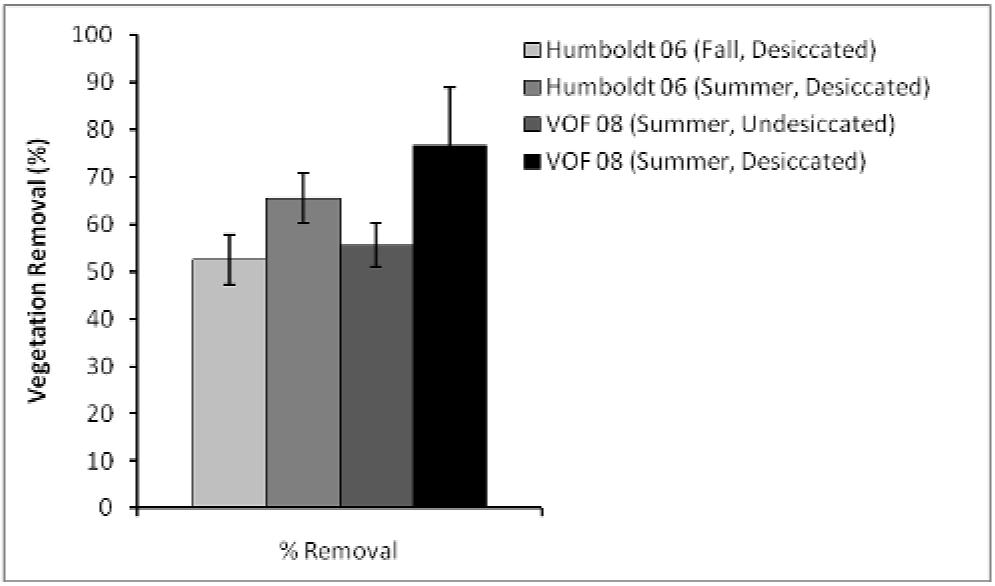
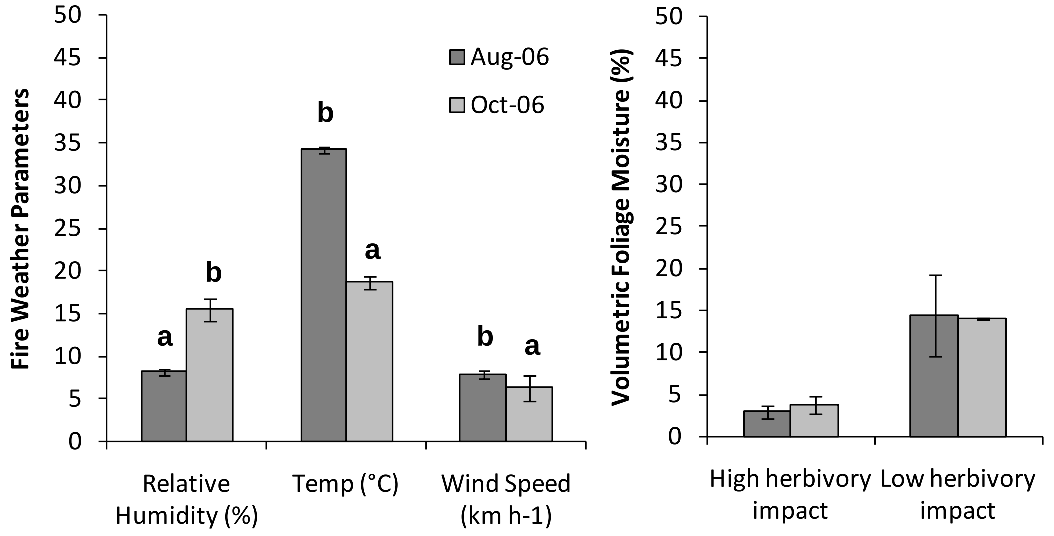


Figure III.6: Fuel consumption by site, burn timing and foliar condition. Vegetation removal indicated by % reduction in vegetation cover obtained from pre and post-fire vegetation monitoring transects (Humboldt N = 18, VOF N = 24). Error bars indicate one +/- standard error. Analysis was conducted on Ln transformed data.

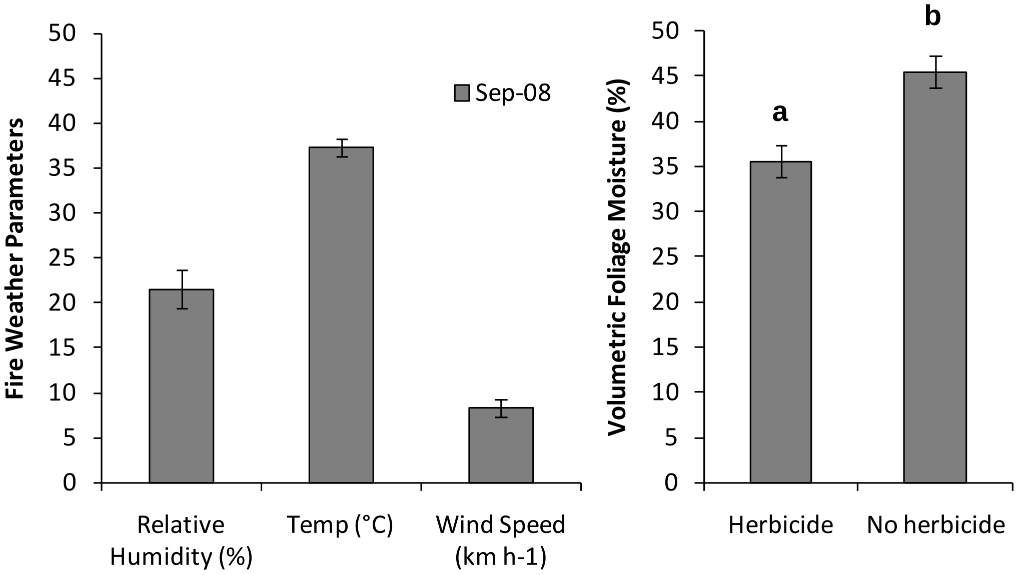
The HB fire weather parameters differed by August vs. October burn seasons (Fig. III.7). Relative humidity during the August fire trials was 7.8% (ratio of partial pressure of water vapor) lower than October ($F_{1,19} = 17.41$, $P = 0.0005$). Ambient temperatures in August were 15.5˚C higher than October ($F_{1,19} = 21.89$, $P = 0.0002$). Wind speeds in August were 2.7 Km h^{-1} higher than October ($F_{1,19} = 18.47$, $P = 0.0004$). Volumetric foliage moisture was 1.3% greater during the October burn than the August burn, 11.5% greater in live in undesiccated (low herbivory) foliage than high desiccated (high herbivory) foliage during the August burn and 10.2% greater in undesiccated foliage than desiccated foliage during the October burn (Fig. III.7). All moisture samples were highly variable. At VOF, fire weather parameters were 21.5% relative humidity, 37.3˚C ambient temperature, and 8.4 km h^{-1} windspeed (Fig. III.7). Volumetric foliage moisture was 9.9% lower in trees treated with herbicide ($F_{1,35} = 7.39$, $P = 0.009$).

Fire behavior data are shown in Fig. III.8. At the HB site the rate of fire spread was 3.9 m min^{-1} faster during August than in October ($F_{1,90} = 4.10$, $P = 0.0458$), and flame lengths were 3.9 m larger during the August fires ($F_{1,2545} = 850.3$, $P < 0.0001$). The VOF rate of fire spread was 5.5m min^{-1} faster in the plots desiccated by herbicide treatment than in the control plots ($F_{1,18} = 2.51$, $P = 0.13$). Flame lengths were 16 m larger in the herbicide treated plots ($F_{1,18} = 8.2$, $P = 0.01$).

The HB maximum temperature was 38˚C higher in August than October ($F_{1,141} = 4.3$, $P = 0.0396$; Fig. III.9). Burn duration was 37.2 minutes longer in August, 107 minutes longer at the surface than the canopy, and 102.1 minutes longer in the litter addition than in litter removal treatments ($F_{23,476} = 10.9$, $P < 0.0001$; Fig. III.9). FII (degree-minutes above 70˚C) differed by burn season, herbivory impact, and thermocouple position ($F_{23,476} = 14.03$, $P < 0.0001$; Fig. III.10). August FII was greater than October, but the increase in surface FII with foliar desiccation due to *Diorhabda* herbivory within each fire season was only a trend (August: $F_{1,71} = 1.75$, $P = 0.19$; October: $F_{1,69} = 2.5$, $P = 0.11$; Fig. III.10). FII was greater at the surface than the canopy during both



a) Humboldt Herbivory Experiment



b) Valley of Fire Herbicide Experiment

Figure III.7. Fire weather parameters by site and burn timing; a) HB or Humboldt Basin, b) VOF or Valley of Fire. Relative humidity, temperature (˚C), and wind speed (Km h^{-1}) were measured at regular intervals during all prescribed burns. HB: N = 23, VOF: N = 6; error bars indicate one +/- standard error; letters (a and b) indicate significant differences ($p \leq 0.05$) among treatments within a site.

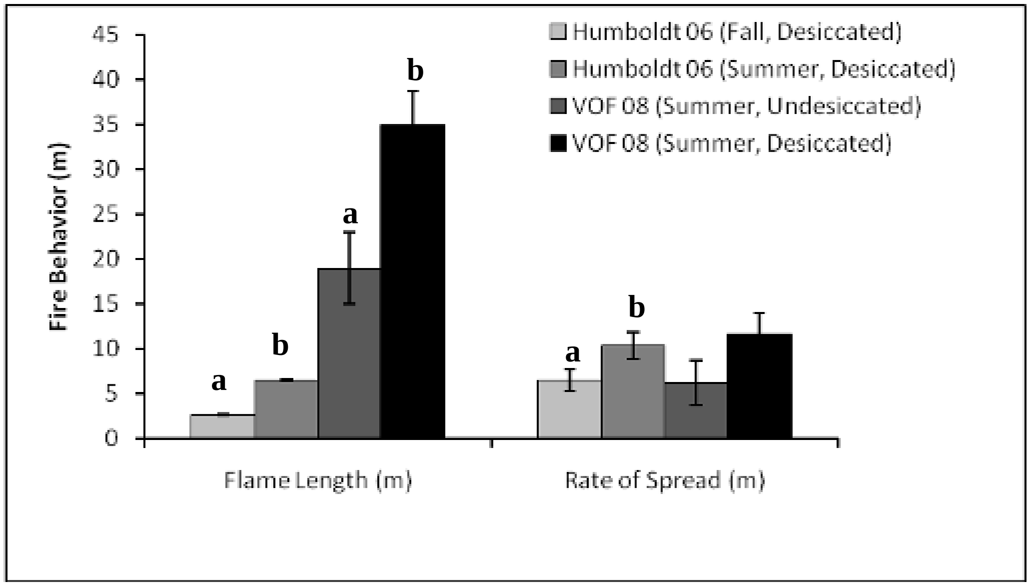


Figure III.8. Fire behavior by site, timing and foliar status. Fire behavior is indicated by rate of spread, flame length, and % *Tamarix* cover reduction. Both Desiccated (herbicide desiccation treatment) and Undesiccated (undesiccated control) foliage conditions were present at the VOF site, while HB only desiccated condition. Values are mean +/- one standard error; HB: N = 2546; VOF: N = 19; +/- indicates the standard error about the mean; a or b indicate differences among treatments within a site at the $p \leq 0.05$ level. Analyses were conducted on Ln transformed data, and arcsine squareroot transformed data for vegetation removal.

burn seasons ($F_{3,519} = 71.12$, $P < 0.000$; Fig. III.10). Litter additions produced greater FII than control or litter removals in both August and October fire ($F_{2,519} = 20.70$, $P < 0.0001$; Fig. III.11).

At VOF, FII was greater in the herbicide treatment ($F_{1,59} = 6.14$, $P = 0.02$; Fig. III.9), but there was no difference in FII between surface and canopy thermocouple positions ($F_{1,59} = 0.30$, $P = 0.58$; Fig. III.10). Maximum temperature differed by herbicide treatment but not by thermocouple position; the maximum temperature of the herbicide treatment was 312˚C higher than the control treatment ($F_{1,59} = 7.15$, $P = 0.01$; Fig. III.9). Fire duration also differed by herbicide treatment but not by thermocouple position; the average fire duration of the herbicide treatment was 57.4 minutes longer than the non herbicide treatment ($F_{1,59} = 5.5$, $P = 0.02$; Fig. III.9).

Green tamarisk samples produced flame 100% of the time, green cottonwood foliage produced flame ~50% of the time, Goodding's willow 33%, and narrowleaf willow 0% of the time ($R^2 = 0.59$, $\chi^2 = 11.8$, $P = 0.0078$, $DF = 14$; Fig. III.12). 'Brown' or desiccated tamarisk samples produced flame only ~33% of the time, while cottonwood produced flame ~67% of the time, Goodding's willow ~33%, and narrowleaf willow 0% of the time, but these samples were highly variable ($R^2 = 0.24$, $\chi^2 = 4.9$, $P = 0.17$, $DF = 14$; Fig. III.12). Tamarisk also ignites more readily than either cottonwood or willows ($F_{18,21} = 2.73$, $P = 0.07$; Fig. III.13).

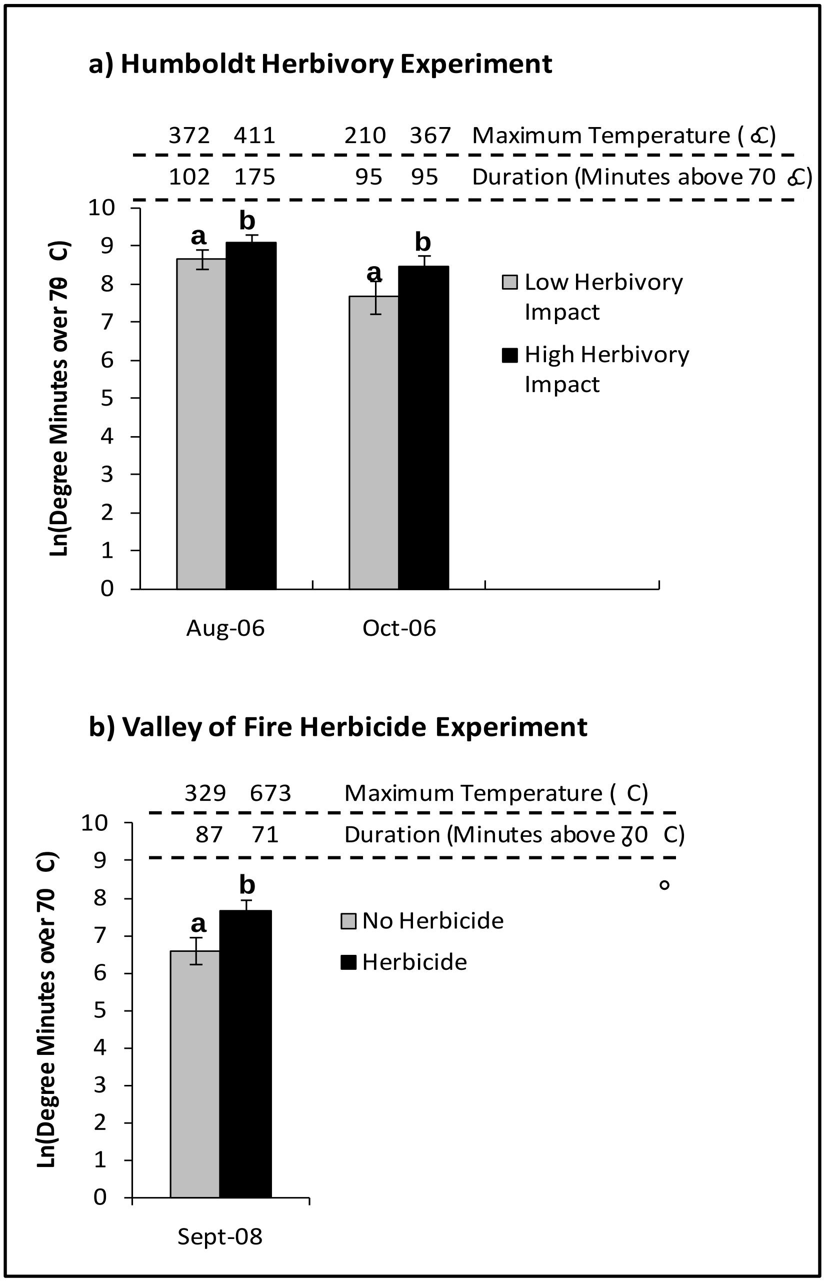


Figure III.9. Fire intensity index (FII) vs. foliage status (herbivory or herbicide desiccation level). At HB, the high herbivory class was defined as > 40% desiccation, and the low herbivory class was < 40% desiccation. At VOF 'Herbicide' is foliage treated to create 'desiccated' condition, and 'No Herbicide' is the control treatment. HB: N= 543; VOF: N = 60; error bars indicate +/- one standard error; letters a and b indicate significant differences ($p < 0.05$) among treatments within a site and burn season. Analyses were conducted on Ln transformed data.

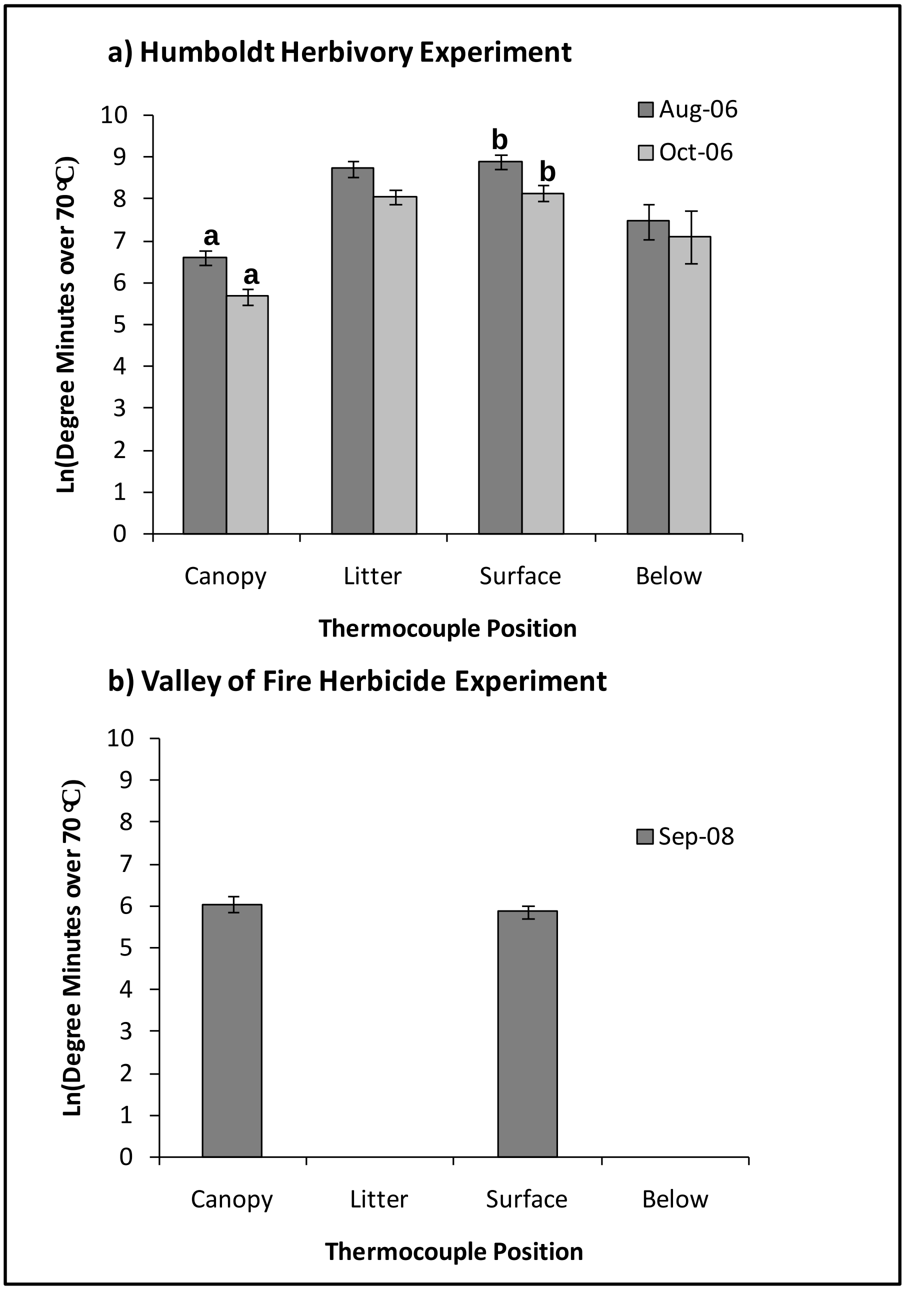


Figure III.10. Thermocouple position by treatment and site, representing vertical profiles of FII in the Humboldt (=HB) summer and fall, and Valley of Fire burn treatments. Thermocouple positions: Canopy = Humboldt at 1m and VOF at 2 m aboveground, Litter = Humboldt only at 7.5 cm aboveground within litter), Surface = at the litter-soil interface), and Below = buried 2 cm below the mineral soil surface). Humboldt: N = 543, VOF N = 60; error bars indicate one +/- standard error; letters a and b indicate significant differences ($p \leq 0.05$) among treatments within a site and burn season. Analyses were conducted on Ln transformed data.

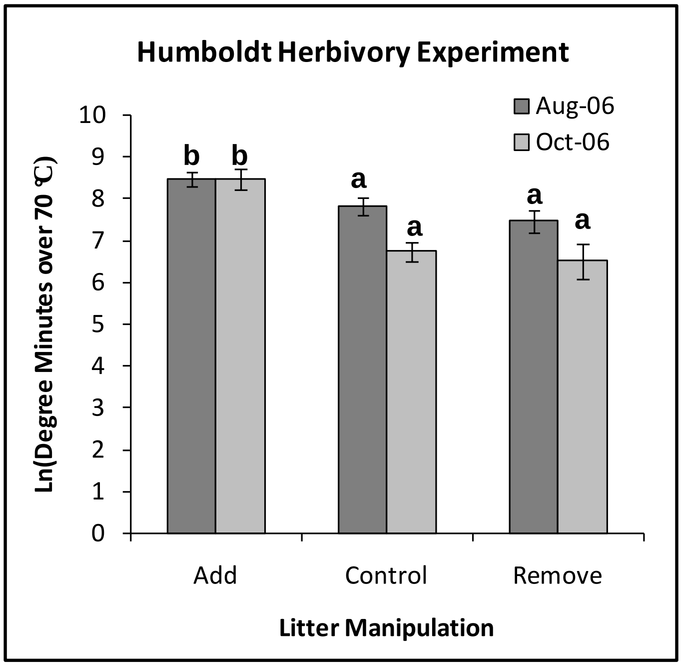


Figure III.11. Fire Intensity Index (FII) in Humboldt (HB) summer and fall burns as a function of litter manipulation. Treatments consist of litter addition (7.5 cm depth), litter removal (bare soil) and an unmanipulated control. N = 543; error bars indicate one standard error; letters a and b indicate significant differences ($p \leq 0.05$) among treatments within a site and burn season. Analysis was conducted on Ln transformed data.

Foliar Flammability Experiments

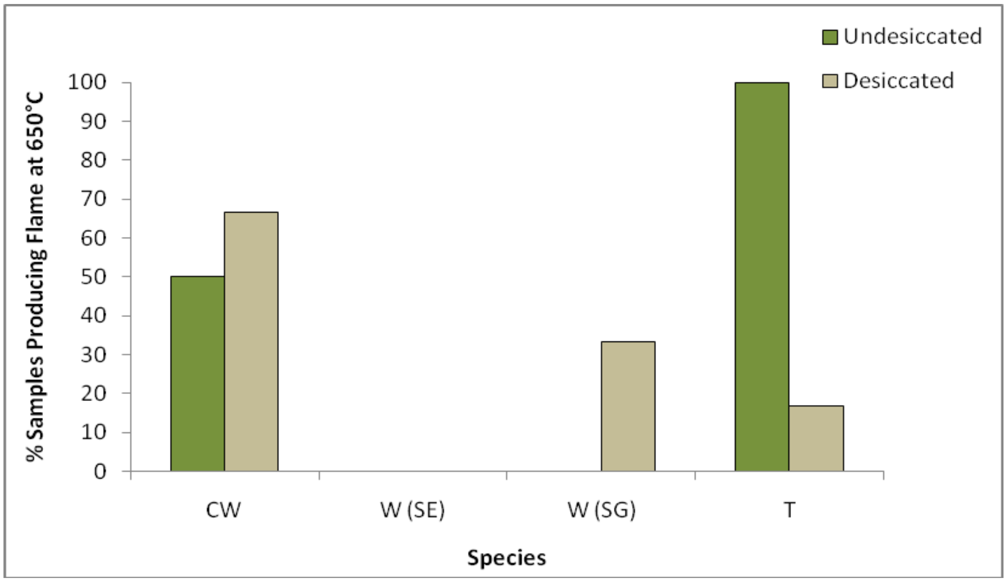


Figure III.12. Percent individual leaf samples producing flame (in addition to glow/char) at 650˚C. N = 30. CW indicates *Populus fremontii*, W(SE) *Salix exigua*, W(SG) *Salix goodingii*, and T *Tamarix* spp. Error bars are absent because the proportions are based on count data.

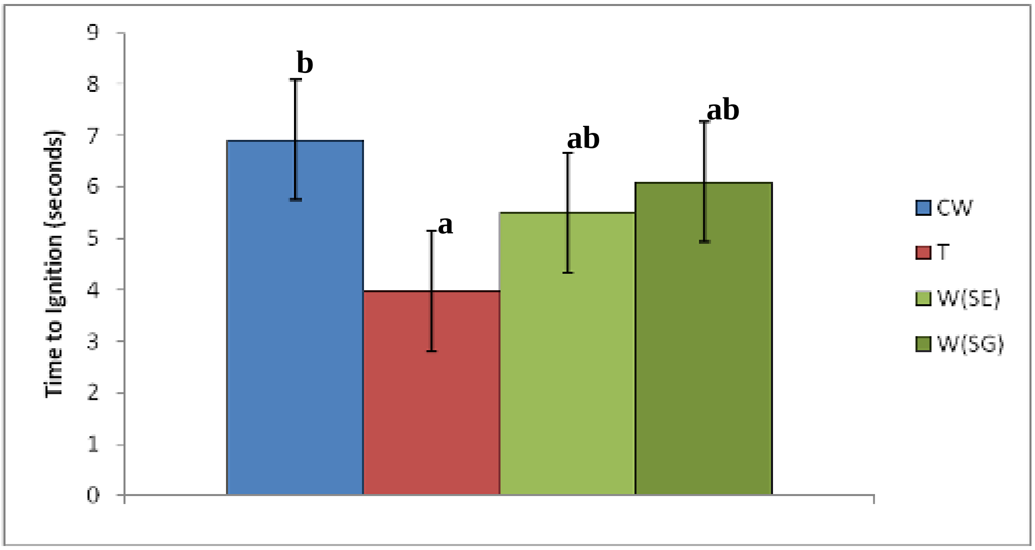


Figure III.13. Time to ignition (flame or char) by species. CW indicates *Populus fremontii*, W(SE) *Salix exigua*, W(SG) *Salix goodingii*, and T *Tamarix* spp. Error bars indicate one standard error; letters (a and b) indicate significant differences among species ($p \leq 0.05$).

TAMARISK AND FIRE
SUB-SECTION III-C. Fire and Tamarisk Mortality

Question addressed: How does *Diorhabda* herbivory and herbicide-simulation of herbivory affect post-fire *Tamarix* dieback and mortality?

Methods

The mortality experiments were conducted at the same study sites (Humboldt = HB; Valley of Fire = VOF), and within the same experimental plots as addressed in Sub-Section III-B so will not be repeated here. At the site currently colonized by *D. carinulata* beetles (HB), we evaluated the relationship between herbivory stress and post-fire mortality, by exploiting existing variability in herbivory impact among trees. Herbivory impact was estimated by dividing defoliated canopy volume by the total canopy volume. Thirty trees were selected per plot in a stratified design where 15 low herbivory impact trees were defined as $\leq$ 60% defoliated, and 15 high herbivory impact trees were defined as > 60% defoliated. At VOF, herbicide was applied a month before the prescribed burn the glyphosate application. The 5 m radius circular plots conducted prior to the VOF burn in the herbicide plots (6 plots total) provided a qualitative assessment of herbicide impact via estimates of foliar desiccation.

At both sites, tree status (live vs. dead foliage), height of re-sprouting plant material, and understory composition were recorded for each monitored tree during a census. Additionally, two 50m brush-belt transects were used per plot to tally the number of live vs. dead individuals to estimate burning-induced plot-level mortality. Mortality was defined as no green foliage or resprouts visible on burned trees for at least 1 growing season post-fire, and follow-up reconnaissance in 2010 indicated that no trees previously recorded as dead had developed any live growth.

Statistical Analyses All statistical analyses were performed with JMP version 8 (SAS 2008) using a critical value of 0.05. Standard error bars were included in all figures for the purposes of visual display, while statistical comparisons were made among sample population means (i.e. treatment plots). Two-factor ANOVA was used to compare mortality by herbivory level and burn treatment at HB. One-factor ANOVA was used to compare mortality by herbicide treatment at VOF. At VOF, one-factor ANOVA was used to compare plot-level mortality by fire intensity. At HB, logistic regression was used separately for each of the treatments (summer burn, fall burn and unburned control) to determine the probability of mortality due to fire intensity.

Results

Diorhabda herbivory enhanced post-fire tamarisk mortality to a greater degree than herbicide-simulated herbivory. At HB, post-fire mortality increased with herbivory levels across burn treatments ($F_{1,10}$ = 11.89, P = 0.01) across burn treatments. However, when burn seasons are evaluated separately, this relationship is stronger in the August burn ($F_{1,5}$ = 6.08, P = 0.07) than in the October burn ($F_{1,5}$ = 0.86, P = 0.41; Fig. III.14). Post-fire mortality increased with fire intensity, as measured by the integrated index that incorporates burn temperature and duration (FII; Drus et al., in press) in the October burn (R^2 = 0.12, χ^2 = 11.4, P = 0.0007, DF = 72), but not in the August burn (R^2 = 0.0028, χ^2 = 0.16, P = 0.69, DF = 70), because overall fire intensity and mortality were higher. These results show that fire intensity and herbivory level are important

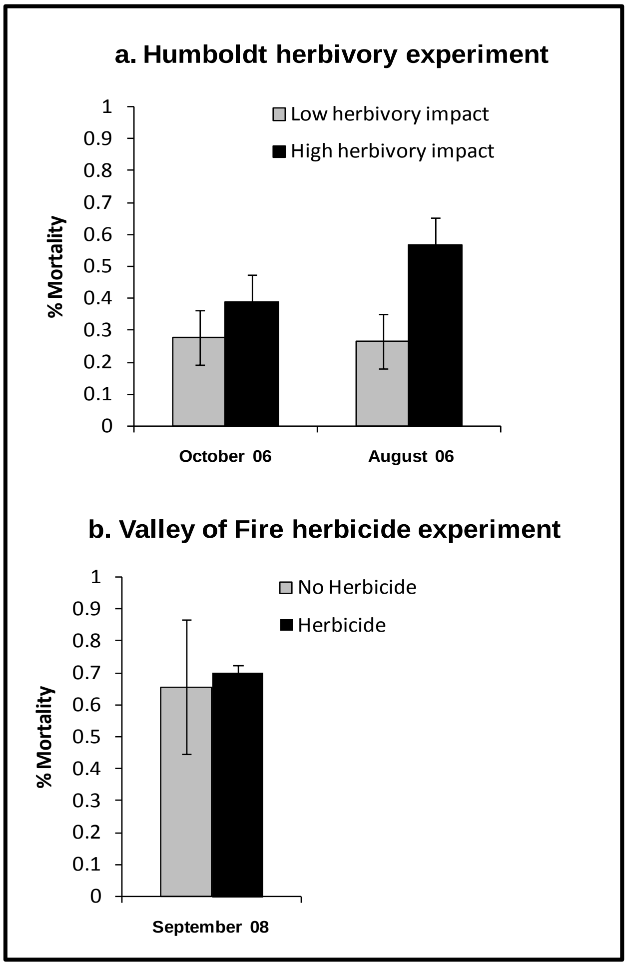


Figure III.14. Percent mortality by foliar status obtained from post-fire resprout monitoring. In Humboldt, proportions were derived from monitored trees at the plot-level ensure differences in foliar status (N = 6). In Valley of Fire, proportions were derived from 2 brush-belt transects per plot (N = 12); error bars indicate one standard error. Analysis was conducted on Ln transformed data.

determinants of post-fire mortality in tamarisk. Higher intensity caused more damage to root-crown tissues and greater subsequent mortality.

However, herbivory stress was the most important determinant of tamarisk mortality because prolonged herbivory made these plants more vulnerable to fire by decreasing the ability to resprout by reducing carbohydrate stores needed to replace damaged tissues (Hudgeons 2007). Burn timing is also important because plant phenology can influence recovery, and weather conditions can influence fire intensity. Carbohydrate reserves fluctuate over the growing season with the lowest levels reached during the height of the growing season (June-August) when all energy is devoted to growth. Therefore, burning in August leaves less energy reserves from which to resprout, corresponding with the higher mortality observed in the August burn treatment. Summer weather conditions promoted higher FII in the August burn, which also contributed to higher overall mortality in the August burn.

At VOF, there was no relationship between mortality and FII ($F_{1,5}$ = 0.01, P = 0.92), or mortality and herbicide desiccation ($F_{1,5}$ = 0.13, P = 0.74; Fig. III.14). FII was enhanced by herbicide treatment, but this increase in FII was not sufficient to enhance post-fire mortality, similar to the August burn in HB. Because herbicide was applied only one month prior to the prescribed burn at VOF, there was insufficient time for physiological stress to develop. Ultimately, post-fire tamarisk recovery and mortality is affected by burn timing and exposure to physiological stress. Differences in the relative influence of herbivory and FII between treatments at the HB site (herbivory > FII August, and herbivory < FII October) suggest that post-fire tamarisk mortality is more greatly influenced by a combination of physical and physiological factors (e.g. FII and herbivory stress), which likely interact. Therefore, for the combination of herbicide and fire to be successful, sufficient physiological stress must be generated through repeated herbicide application, or a single application with a high kill rate.

Overall Conclusions – Tamarisk and Fire

Section III-A Regional Survey of Tamarisk-Fire Relations

- Native tree fuel consumption and mortality increased as a function of tamarisk density in burned riparian sites across the U.S. southwest, indicating that tamarisk burns more intensely than native vegetation and causes greater damage to the ecosystem.
- Native species are less able to recover from fires as the proportion of vegetation comprised by tamarisk increases, further indicating that it poses severe threats to remaining native vegetation.

Section III-B Tamarisk Condition and Flammability

- Fire intensity increased marginally with defoliation intensity in tamarisk, but this temporary increase is outweighed by the potential for herbivory by *D. carinulata* to reduce fuel availability over the longer-term.
- A greater proportion of live (green) tamarisk foliage produced flame than willow or cottonwood foliage, while a lower proportion of desiccated tamarisk foliage produced flame than willow and cottonwood, possibly suggests that native riparian vegetation becomes more flammable under prolonged drought conditions.
- Live tamarisk foliage ignites (chars or flames) more quickly than live native fuels, a physical mechanism contributing to the greater overall flammability of tamarisk-invaded riparian areas.

Section III-C Tamarisk Mortality

- Prolonged defoliation by *D. carinulata* can cause mortality as metabolic reserves are depleted, and fire enhances weed mortality by interacting with physiological stress caused by herbivory.
- Herbicide-induced defoliation did not result in tamarisk mortality, as this short-term treatment was only intended to simulate the direct effects of herbivory, not its long-term effects on plant health.

Given the widespread displacement of native vegetation in desert riparian ecosystems by tamarisk invasion, fire is likely a permanent physical disturbance factor that will become more frequent and damaging as invasive plant density increases. Biological control provides the opportunity to reduce the availability of fuels and enhance post-fire tamarisk mortality. Reduced tamarisk recovery may allow passive restoration as native plants recover more readily, and should also open habitat space for active restoration practices where appropriate.

OBJECTIVE 4: *Evaluate herbicide and cultural treatments for the control of Russian knapweed, a recent invasive species that responds positively to disturbance and will function as a surrogate for treatment of other secondary weed invasions.*

CHAPTER IV. Effectiveness of herbicide treatments for control of Russian knapweed (*Acroptilon repens*) as a secondary invasive weed in southern Nevada

Background

A key management concern in protected wetlands and associated rangelands of Clark County and adjoining regions is invasion by non-indigenous plants, of which there are approximately 72 genera (90+ species) of concern in Mojave Desert riparian systems (Dudley 2009). About 36 of these are considered of moderate to high impact, and the majority are weedy forbs, annual or biennial species which can form extensive, low-growing infestations. Although tamarisk (*Tamarix* spp.) is probably the most serious invasive plant in the region, particularly in riparian ecosystems, many others occur at varying densities and often in association with tamarisk (Shafroth et al. 2008). Thus, they potentially can become more serious environmental weeds when the dominant invasive plant is removed (see Section 1, this report). This is partly owing to their co-occurrence with other weedy plants like tamarisk in degraded ecosystems, such that removal of one opens space for the others to expand populations as a consequence of reduced competition. Another major reason is that management actions to remove a dominant weed, particularly tamarisk, typically involves physical disturbance of the soil which facilitates secondary invasion of other weeds that can take advantage of such conditions (Hobbs 1991, D'Antonio and Meyerson 2002). From a management perspective, it is important to rapidly develop protocols for treating such secondary invaders before they become serious problems in their own right, and in particular before they produce a large seed bank that subsequently creates long-term management problems.

Many of the major secondary invasive plants are forbs that readily occur in the tamarisk understory (Section I, this report), such as *Lepidium latifolium* (perennial pepperweed/tall whitetop), *Salsola* spp. (Russian thistles) and an increasingly problematic species is *Acroptilon repens*, (a.k.a. *Rhaponticum repens*) or Russian knapweed. Russian knapweed (referred to here as RK) is a noxious, perennial broadleaf herb occurring throughout western and central North America from Mexico to southern Canada (Carpenter and Murray 1998). This plant maintains a long-lived, rhizomatous root system and forms dense stands typically about 3 to 6 dm tall, and sometimes over a meter tall. Clonal stands can inhibit establishment of desirable plants, both in wetland meadow-type ecosystems and agricultural fields, particularly alfalfa. Because it is important in agriculture and rangelands, RK has been a target for several weed control studies using a range of herbicides (as described below). In contrast there are few such studies in wildlands or managed natural areas, and the application of herbicides treatments must be evaluated in a different manner than would be done in agricultural sites because of the sensitivity concerning pesticide impacts to native organisms. Also, many herbicide trials targeting RK only used a single chemical, perhaps at varying concentrations, so it is difficult or impossible to evaluate the comparative effectiveness of different herbicides in establishing protocols for a given area. Furthermore, RK is a fairly recent invader in southern Nevada, so there are no data indicating what herbicides or treatment conditions are best suited to its control in warm desert regions.

This evaluation of weed control treatments against RK is part of a larger program to develop best management practices for tamarisk control, particularly in light of the recent establishment of biological control of as a weed management approach. Biocontrol of Russian knapweed has also generated substantial interest in the Western states, but that program is much less developed. Potential agents include insects, mites (*Aceria acroptiloni*), fungal pathogens and a specialist nematode (*Subanguina picridis*) that is an internal parasite to weaken target plants (Schaffner et al. 2001). The mite and the nematode have been approved for open release, but have not been shown to be effective under field conditions, while other agents are far from being at a stage where they can be implemented under open field conditions. An original intent of this project was also to conduct trial releases of agents against *Salsola tragus* (Russian thistle), which co-occurs with RK in these same habitats, but the candidate agents did not receive regulatory approval within the project timeframe (Smith et al. 2010). Hence, we must rely on traditional methods for control of these weeds, generally involving herbicidal applications, until biocontrol agents can be made available. Handpulling or other mechanical methods are not effective with RK due to the extensive underground rhizomatous root system. RK also readily resprouts after fire and grazing. Herbicide application is likely the ideal method to control RK in southern Nevada due to its limited distribution and total acreage. If effective chemical applications can be developed through this study then it will be feasible to eradicate RK from Southern Nevada.

One area where RK is increasing in abundance and propelling increasing concern over its potential to negatively affect native vegetation and associated wildlife is at Ash Meadows National Wildlife Refuge just north of Clark County into Nye County, NV. Incipient populations are known to occur within Clark County along the Muddy River and at isolated areas in the Spring Mountains, but it does not yet achieve detrimental levels. RK is almost certain, however, to become a more serious invasive plant problem in the future, as it is already designated as noxious in other states at similar latitudes, such as New Mexico and Texas, as well as in northern Nevada.

Once RK populations are well established they become difficult to control without multiple year commitment due to its extensive underground biomass. Thus, it would be important to know ahead of time what herbicide treatment approaches may be effective so that control can be implemented before damaging weed levels are met. Several herbicides known to be relatively safe for environmental applications and non-toxic to wildlife are available for potential control of RK and other environmental weeds, particularly where they are invading following treatments of other weeds, particularly tamarisk. These include compounds such as imazapyr, aminopyralid, imazapic and chlorsulfuron, which are the subjects of this effectiveness evaluation study. All are considered to be broad spectrum in activity and are intended for rangeland or wildland application against broad-leaf weeds, including several of the knapweeds (*Centaurea* spp.) common in such situations.

Study Site and Methods

This study was conducted at Ash Meadows National Wildlife Refuge, where efforts to control tamarisk are well-established and densities are being strongly reduced within and near the Refuge. Substantial stands of RK are also present on the refuge and at densities conducive for this study with large enough populations to allow for replicated experimental block designs. RK is a challenge and concern at Ash Meadows because it is tolerant of wildfire and colonizes after ground disturbances following stream restoration activities at the Refuge. It initially established mostly in abandoned agricultural fields and has persisted, aggressively spreading into adjacent spring-fed wetland areas throughout the Refuge.

The infested area was initially divided into 6 blocks of treatment plots with each block separated by an area greater than the width of the block itself to provide a barrier between the block. Then, each was sub-divided into 7 treatment plots for experimental application of herbicides to 6 plus a control plot. Each treatment plot measure 6 by 12 meters, with a buffer strip of at one meter between each. Two treatment replicates were in each block yielding 12 replications per treatment, and totaling 0.12 acres for each treatment.

For each plot, vegetation cover, stem density and biomass was measured for each plant species present, within 3 quadrats of 0.5 meters on a side. Each quadrat was randomly chosen, using a PVC frame placed at co-ordinates chosen a-priori. Initial sampling took place in Fall of 2005 when plants were close to maximum biomass (no longer growing) and easily identified.

Herbicide treatments were applied in February of 2006. The treatments were implemented by the National Park Service-Lake Mead Exotic Plant Management Team (EPMT) under the direction of Curt Deuser. Temperatures were mild (ca. 14°C) with 51-75% cloud cover, and relative humidity was 31%. Wind was from the west at 1-9km/hour with average 3km/hour, sufficiently low that spray drift was minimal. Herbicide was broadcast foliar application with a $C0_2$ pressurized backback boom sprayer with six nozzles calibrated to apply 20 gallons per acre (each plot received 0.4 gallons of spray mix). At the time of application RK cover was near 100% and its phenology was dormant with senesced shoots.

Treatments[1]:

1. Habitat® (active ingredient: imazapyr) at recommended rate of 16 oz per acre with .25% v/v Kinetic (Helena) surfactant. Each plot received 9.5 ml of Habitat and 3.2 ml of Kinetic.
2. Milestone® (active ingredient: aminopyralid) at rate of 3 oz per acre with .25% v/v Kinetic Surfactant. Each plot received 1.8ml of Milestone and 3.8 ml of Kinetic.
3. Milestone® at 5 oz per acre with .25% v/v Kinetic (Helena) surfactant. Each plot received 3ml of Milestone and 3.8 ml of Kinetic
4. Milestone® at 7 oz per acre with .25% v/v Kinetic (Helena) surfactant. Each plot received 4.1ml of Milestone and 3.8 ml of Kinetic
5. Plateau® (active ingredient: imazapic) at recommended rate of 12 oz per acre with 32 ounces of MSO per acre. Each plot received 7.1ml of Plateau and 20.7 ml of MSO.
6. Telar XP® (active ingredient: chlorsulfuron) at rate of 1.5 oz per acre with .25% v/v Kinetic (Helena) surfactant. Each plot received 0.84grams of Milestone and 3.8 ml of Kinetic.
7. Control – no herbicide applied.

[1] *Trade names are for identification purposes only, and are not intended as endorsements*

The aminopyralid treatments were conducted at several concentrations that bracketed the recommendation range of application rates, as we were interested in establishing a more detailed assessment of this herbicide because it was relatively new, and offered good promise as a wildland weed treatment.

Vegetation within plots were re-sampled in late May/early June 2007 in the same manner as pre-treatment to evaluate species responses. At that time additional plots were laid out to expand the treatment types in the Fall of that year, and definitive results were to be determined in 2008. However, private applicators under contract from the Refuge conducted chemical treatment of the whole experimental area during the summer of 2007 and disrupted the experimental set-up, along with any potential for examining any further results. Thus, results presented were only from the season following initial treatments, and while we considered these results to be preliminary, they still provided a fairly clear series of responses to treatments.

Results

Pre-treatment vegetation traits did not differ across treatment plots based on means for each treatment, so for simplicity, herbicide response results are shown as direct comparisons of the treatments themselves rather than changes pre- to post-treatments. All herbicide types and dosage rates appeared to reduce the vegetative cover and plant abundance of RK (Fig IV.1). Aminopyralid at all applications rates clearly and strongly depressed RK in those plots to roughly 50% to 15% of cover and densities in untreated control plots, although the impacts were not definitive for the herbicides imazapyr, imazapic and chlorsulfuron. Higher aminopyralid dose levels at the upper end of the recommendaed range of application rates (5 and 7 ounces per acre) had greater impacts than the lower end of the range (3 ounce), while there was an interesting but probably not substantive trend for the medium application rate (5 oz.) to yield the highest efficacy for weed control. With minor variation, these trends were the same for both RK cover and plant density.

The summer 2007 disruption of the experimental plots by Refuge staff or contractors caused us to terminate this experiment prematurely, so there are no data for the intended final sampling date when we anticipated the most substantive responses, nor for associated plants, both native and non-native.

Interpretation and Implications

Despite the pre-mature termination of this study, it is still reasonably clear that an herbicide using aminopyralid as the active ingredient provided superior weed control of Russian knapweed during the first post-treatment growing season at the test site. First year results that indicate clear in efficacy are likely to show consistently better effectiveness in subsequent seasons, particularly with re-treatment using the same product. Ash Meadows is located within the broad ecotone between the Great Basin and Mojave Deserts, and seasonal conditions are similar to those experienced throughout much of Clark County, so we would extend a recommendation that RK (and likely other weeds in the family Asteraceae) may be suited for control with this product. Similar trials were carried out in New Mexico and Colorado with the same compounds, with less clear results presumably owing to weather conditions and phenological stage of the target weeds at time of treatment, but were generally consistent with these findings (C. Deuser, personal communications).

Aminopyralid is a broad spectrum herbicide intended for control of annual, biennial and perennial broadleaf forbs in rangeland environments that has both systemic/post-emergent and seasonal residual control potential. Thus, it may help control weeds in several life stages, however caution should be applied because its effects carry over the course of several months, rather than readily breaking down, so it can potentially affect other plants during the season. Aminopyralid does not, however, have substantial effect on grasses, which are often the desired species in many range and pasture systems. Its activity can extend to shrubs and woody plants, however according to label recommendations control is not high and other compounds are suggested for addition to control such weeds (Enloe et al. 2008). It is effective against true thistles (Bukun et al. 2009) which frequently co-occur with RK.

Aminopyralid is not federally restricted, and has been shown not to have impacts on grazing animals, with very low toxicity to birds, fish, mammals and aquatic invertebrates. It breaks down into normal soil components and is applied at fairly low rate so run-off is considered minor, and also breaks down within 24 hours in water. Aminopyralid has been shown to affect vegetable crops when present in manure used as fertilizer (Dow AgroScience 2007), but this should have no bearing on environmental use of the herbicide. The primary caution is that it is active across many plant taxa, particularly broadleaf species, so incautious use is likely to affect desirable plants in close proximity.

The other compounds, including imazapic, imidazolinone and chlorsulfuron gave lesser efficacy but are still widely used for controlling rangeland weeds. It should also be noted that these compounds may have been more effective if applied during different plant phenological stages. Imidazolinone compounds are useful for control of a similarly wide range of weeds, including noxious grasses while having little effect on some of the more desirable warm-season grass species. For example RK treatments using imazapic in northern Nevada has little or no impact to Great Basin wildrye (Schulz 2005). It can also be used as a pre-emergent. The latter trait is of little consequent for RK control, as it spreads primarily by clonal expansion and there is rarely need to use a pre-emergent to reduce germination since it rarely spreads via seed Benz et al. (2005). It also is not recommended in or near water, so application in riparian and wetlands systems seems largely inappropriate. Chlorsulfuron is most typically used on perennial weeds, so is appropriate for RK control although it also gave poorer efficacy than the aminopyralid treatments. It can, however, be used near water or in seasonally wet locations with low risk of contamination.

Imazapyr compounds, on the other hand, are routinely used directly in aquatic weed applications, and are also particularly important in wildland applications for forbs as well as woody plants, often including tamarisk. It does have residual activity in the soil for up to 20 weeks, so caution again is advised if other plants are at risk later in the season. It is of extremely low toxicity to wildlife and invertebrates, the mode of action involving a biochemical pathway that does not exist in animals. Based on existing information and particularly on the results from this study, aminopyralid remains the preferred choice for RK and likely other secondary weed control in this region.

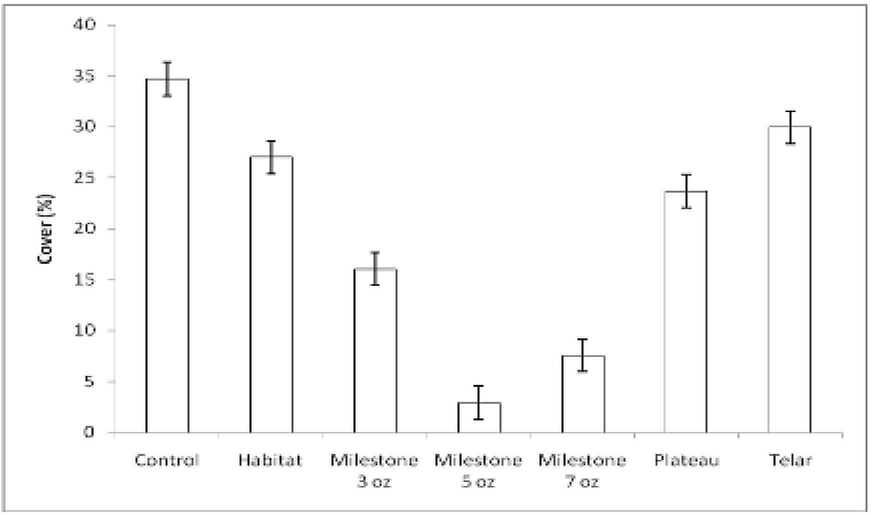
Other herbicide treatments have been evaluated for the control of RK in rangelands. For example, Laufenberg et al. (2005) tested a variety of compounds and found that clopyralid plus 2,4-D gave acceptable control while neither glyphosate nor fosamine had significant impact on weed densities. That treatment is probably not advisable in wildland or wetland situation because of the known toxicity of both 2,4-D and clopyralid compounds (http://www.mindfully.org/Pesticide/Clopyralid.htm). These authors indicated that revegetation was advisable in infested areas, along with re-treatments, to enhance potential for recovery of native vegetation where native seedbanks have been depleted by many years of weed dominance. Enloe et al. (2008) compared several agents for RK control in Great Basin rangelands and showed that, similarly to this current study, aminopyralid provide good control that was comparable to that achieved with picloram + 2,4-D amine whereas clopyralid and imazapic yielded poorer performance; again, its lower toxicity combined with good target mortality indicates aminopyralid as a preferred option.

We caution again that these are preliminary results, based on one season of observation and no re-treatments, which are advisable when managing RK and other rangeland and wetland weeds. However, in practice an herbicide shown to be effective in initial trials will almost always be the preferred treatment in follow-up treatments, so aminopyralid would likely remain the appropriate method used under these conditions.

Conclusion

Testing of four widely used herbicides for managing invasive Russian knapweed showed that aminopyralid, available under the trade name Milestone®, was the most effective compound for controlling this clone-forming, perennial weed near wetlands during the first year of treatment. Because it is an abundant secondary invasive plant associated with tamarisk in many arid regions, this is a recommended treatment in association with tamarisk control efforts, whether that involves traditional chemical/mechanical control or biological control. This weed is favored in disturbed soils, so it is likely to be a factor in tamarisk management in Clark County in the near future. Likewise, current secondary invaders such as the *Centaurea* knapweeds and numerous other weedy forbs are within the target range of this herbicide, so until non-chemical mechanisms of weed control are available for secondary invaders, aminopyralid appears to be the preferred treatment method for weed control in riparian and marshland areas.

a.



b.

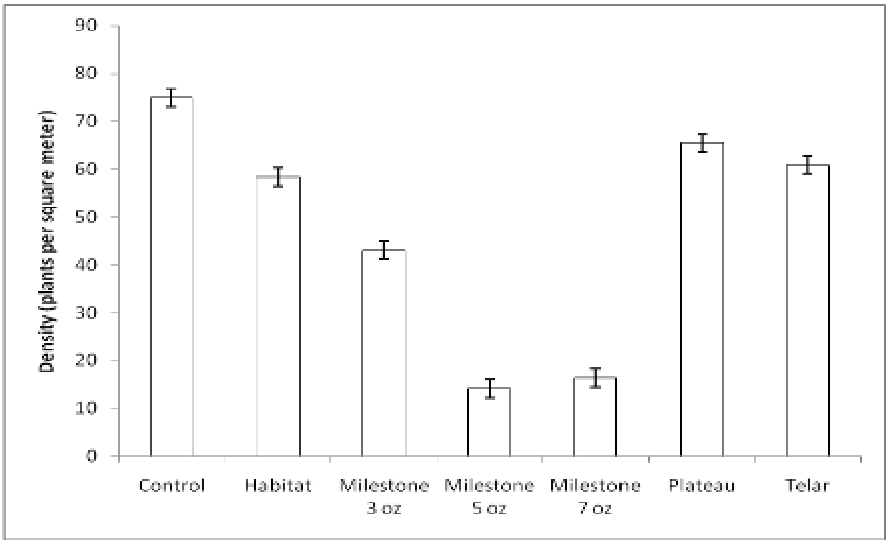


Figure III.1. Russian knapweed percent cover (a.) and densities (b.) during Spring 2007 vegetation sampling 7-8 months following treatment applications; error bars indicate 1 standard error about the mean. Because these were intended to be preliminary data, statistical analyses were not performed.

OBJECTIVE 5: MONITORING BIOLOGICAL CONTROL OF TAMARISK

OBJECTIVE 5 *Monitor and evaluate the process and effects of tamarisk biocontrol on ecosystem function and biodiversity, including:*

5-A *Monitor dispersal and establishment of Diorhabda carinulata, document life cycles and life history adaptations, including genetic changes, in the southern Nevada latitude. Monitor predators that can inhibit, or respond to, Diorhabda establishment.*

5-B *Monitor effects of Diorhabda on tamarisk condition and vegetative architecture, defoliation-regrowth dynamics and responses by associated vegetation, wildlife and soils to tamarisk defoliation. Assess fire risk dynamics related to defoliation.*

SECTION V-A. Biological Control Assessment in Clark County and Adjacent Regions

Background

The 2001 introduction of the tamarisk leaf beetles (*Diorhabda* spp.) for control of *Tamarix* spp. North America provided the justification to start planning for eventual introduction and/or dispersal into Clark County riparian and wetland ecosystems, particularly the Virgin River and the mainstem Colorado River. Our original CC-MSHCP project included simulating the effect of this specialist herbivore to evaluate responses of vegetation composition and structure, fuels and fire risk, and wildlife habitat associations, once biological control with *Diorhabda* (biocontrol) was implemented in southern Nevada. During the course of this project, however, *Diorhabda carinulata* that had been introduced by resource managers into the Virgin River watershed in St. George, UT, proceeded to disperse unassisted in multiple directions, including along the Virgin River in Arizona and Nevada, as well as southward to the Colorado River where it is now present within the Grand Canyon (Fig. V-1).

This changes the nature of any future tamarisk management, as biocontrol will inextricably be a factor in weed management and system responses. The original project was to evaluate the effectiveness of prior tamarisk treatments in promoting recovery of vegetation to support Covered and other sensitive wildlife species, but biocontrol was not intended as a weed control method to evaluate directly. This unanticipated insect release provided the context and need for monitoring its impact and effectiveness as a weed management 'tool' for invasive plant suppression and wildlife habitat restoration. To address this situation we assembled a team of natural resource specialists from academic units, and State and Federal agencies, to construct an extensive and detailed monitoring program, supported in part by an amendment to the current project, and by funding resources developed by research partners.

As of this reporting date, we are still early in the process of *D. carinulata* dispersal, establishment and impact in the Clark County region, but the data being generated will prove to be a critical information base that is largely or entirely lacking in all other locations where tamarisk biocontrol has been introduced. The multi-agency, multi-disciplinary monitoring program that we have put into place, including surveys of birds, small mammals and bats, reptiles and amphibians, arthropods, water resources and other ecosystem elements (Bateman et al. 2010) will also provide the scientific framework for a larger program facilitated by the Tamarisk Coalition (Dudley is a former Board member of this NGO). The program is to be supported by the Walton Family Foundation along with other leveraging partners, to implement riparian habitat restoration throughout the Colorado River Basin for the immediate purpose of enhancing native habitat suitable for supporting the southwestern willow flycatcher (*Empidonax traillii extimus*) as well as for other federally listed and/or regionally sensitive wildlife species. The restoration program is initially focused on the Virgin River watershed as the only place where *Diorhabda* spp. beetles are found within the breeding range and designated Critical Habitat of the endangered flycatcher sub-species.

The presence of *D. carinulata* in southern Nevada also raises questions about how this insect will respond to the Mojave Desert environment, as there are strong differences between life cycles and other basic biological features across the geographic range of its introduction (or the introduction of sub-species or conspecifics), particularly given the variety of thermal, photoperiod and other environmental variables presented (Tracy and Robbins 2009, Bean et al. 2007, Dalin et al. 2010) . Basic questions involve when insects are active, how many generations are produced in a season, when do they enter diapause, and what are the effects of the herbivore on target plants in this region. Results so far are not definitive because there is considerable variation related to the colonization process itself, and furthermore there is strong indication that this population in Nevada has already experienced considerable evolutionary selection that makes patterns difficult to distinguish (Bean et al., submitted). Therefore, the collected life history data are preliminary, and intended to provide the baseline for assessing biocontrol dynamics and host plant impacts as the population because more solidly established in the region.

Methods

Diorhabda censusing and impacts Beginning in 2006 the process of *D. carinulata* establishment and dispersal was monitored, initially by simple sweep-net and visual observations from representative *Tamarix* plants, using methods developed at other biocontrol research sites to assess abundances of life stages on plants at given locations (Dudley et al., in press, Pattison et al. 2010). In 2009 a more standardized protocol was developed to provide consistent record of the establishment process, life cycles and dynamics of colonization, as well as to document impacts to host plants, associated organisms and other data that might clarify the effectiveness of this weed control method. In Spring 2009 five plants were chosen semi-randomly for regular monitoring at each of 8 locations in the lower Virgin River watershed within Arizona and Nevada; some sites were continued in 2010 while others were dropped or added based on practicality of regular sampling and to cover the full extent of the drainage. Each plant was measured (average height from 3 measurements, width), its location entered by handheld GPS device, and other ecological variables noted. In addition, the informal monitoring was continued for other sites in the Utah portion of the watershed, including the Santa Clara River as well as the mainstem Virgin. Additional sites in other locations were occasionally sampled to provide a comprehensive record of where colonization occurred, such as La Verkin Creek, upper forks of the Virgin River, Ft. Pearce Wash, Paria Canyon, Beaver Dam Wash, upper and lower Meadow Valley Wash, White River and Muddy River, Warm Springs NWR, Las Vegas Wash and numerous other tamarisk-infested locations in Clark County and adjoining regions.

The regular monitoring stations were sampled bi-weekly during the active growth season. At each station, visual counts were first made by counting insects of each stage (egg masses, 1st and 2nd instars, 3rd instar, adults) on 2 branches roughly 40cm in length on each of the 5 plants. Predators and other visible arthropods and other organisms were also surveyed. Then, five 1-meter sweeps with standard sweep net were conducted on each plant, and captured insects counted, recorded and released (*D. carinulata* and other taxa). The conditions of the target plants was noted on each sampling date, including percent of tree with foliage present, proportion of that foliage that was healthy-green, yellow (senescence or damage from the tamarisk leafhopper, *Opsius stactogalus*), dry-green or brown representing beetle defoliation, and green re-growth foliage; percent of the tree volume that appeared live vs. dead (based on amount of dead wood) was also recorded.

In 2010 a more intensive sampling regime was instituted in order to provide information on both arthropods associated with different vegetation types, including *Tamarix ramossisima, T. parviflora, Prosopis* spp., *Populus fremontii, Salix* spp., *Pluchea sericea, and Salsola* spp. This was to indicate the quality of each vegetation type in terms of food resources available for insectivorous wildlife, and in particular to evaluate whether invertebrate abundances on Tamarix spp. vs. the other plant types changed over the course of *D. carinulata* establishment (hypothesis being that these beetles would, in turn, support generalist arthropod predators that would enhance the overall resource quality of this habitat type). These data are not presented here because they are preliminary, but are archived as part of this project for future comparison.

To test for the relationship between *Diorhabda* development and daylength, which previous studies have shown to induce reproductive diapause prior to onset of winter (Bean et al. 2007), additional collecting surveys were done at 8 set locations in the Virgin, Santa Clara and Muddy Rivers. This involved sweep net capture of a minimum of 25, and usually 100+ individual adult beetles at each station (if present), then immediate freezing and shipping to the Palisade Insectary in Colorado where we dissected males and females to determine diapause condtion (Bean et al. 2006). These collections continue in 2011.

Results and Interpretation

Biocontrol establishment in the Virgin River watershed As illustrated in the series of maps comprising Fig. 2, *D. carinulata* beetles have moved from the immediate vicinity of St. George where it was released in 2005 and remained locally established for 2 years, to points approximately 100 km (60+ miles) distant over the course of the subsequent 3 years. The population entered an epidemic dispersal phase in 2008 when beetles moved large distances, entering Arizona downstream into the Virgin Gorge as well as a long-distance dispersal event that brought beetles for the first time into Clark County in the upper portion of Meadow Valley Wash.

Major tamarisk defoliation occurred downstream in the Virgin River to Littlefield AZ in 2009. In 2010 beetles along with defoliation were documented on the mainstem Virgin River within Clark County, initially within the City of Mesquite, and over successive generations the main population and full defoliation terminated in the immediate vicinity of the Highway 170 bridge over the river at ‘Riverside’, with isolated sub-populations of beetles extending downstream to approximately 2 km below Meadowlands Farm. One of these sub-populations was immediately adjacent to the site where collaborator Ben Conrad and others associated with DRI and UNLV are monitoring changes in landscape-level evapotranspiration from tamarisk stands as herbivory by beetles reduces live foliage cover (see Appendix II).

The Meadow Valley Wash population was fairly stable in 2009, then rapidly dispersed downstream during 2010 after building up a large population fed on a major tamarisk infestation adjacent to the largely-abandoned town of Carp, first entering the Moapa Valley in July. By September 2010 this population had crossed Interstate 15 near the confluence with the Muddy River, and the southernmost population extent was in the town of Logandale (Fig. V.2). We have maintained *D. carinulata* ‘pheromone traps’ (a collaborator, R. Bartelt, USDA-ARS, has produced a synthetic chemical that mimics the chemicals the adult beetles use for attracting mates and maintaining aggregations; Cosse et al. 2006) at many of these locations to detect when beetles arrive in different parts of the region, while the traps maintained at the Moapa Valley/Warm Springs Reserve Complex have not indicated beetle arrival at this location during 2010. Beetles did colonize this site, which formerly supported southwestern willow flycatcher (SWFL) prior to the destructive, tamarisk-fueled fire of July 2010, in Spring of 2011 but no defoliation is likely to occur during the SWFL breeding season.

Flooding in December 2010 certainly caused major losses of the over-wintering beetle populations, but based on Spring 2011 censusing, we anticipate that both *D. carinulata* populations (Muddy River/Meadow Valley Wash and Virgin River) will converge at Lake Mead late during 2011 growing season, or possibly in 2012 if population recovery from winter flooding is slow. The population in the Grand Canyon is also derived from the beetle introductions into St. George, having dispersed down Paria Canyon to the Colorado River; collaborators will be monitoring the progress of this population as it approaches Lake Mead from the east (M. Johnson, Northern Arizona University, pers. comm.).

At least two generations of the *D. carinulata* are produced in a single growth season, and there is evidence of a partial third generation or cohort in the fall but that is not consistent across sites (Fig. V.3). This bivoltine pattern can be seen n the first graph of Fig. V.3 (Cedar Pocket 2009), the beetles that overwintered at the site as adults (they colonized this site in 2008) emerged from the litter in late April as indicated by the first peak in adult numbers in early May. Those over-wintering adults then naturally died, followed by their larval progeny feeding during May and early June to emerge from pupation in June and the adults were present for several weeks into late July. The second cohort reached peak density in late August, although larvae were not as numerous as in the first generation presumably owing to less green tissue available; the high adult density reflected aggregation where green material was still present. The following year (2010) indicates a response fairly common at biocontrol sites in other regions in which densities do not reach the same high levels, a consequence of reduced tamarisk foliage as well as a possible increase in the numbers of arthropod predators that retard population growth (Dudley et al. 2008).

The other Virgin River sites were not colonized until late 2009 or 2010, so this life history pattern was similar but over too short a period of time to resolve clear patterns in population dynamics. For example, ‘Big Bend’ approx. 18 km downstream did not show strong increase in *D. carinulata* numbers until the single cohort in late summer 2010 (Fig. V.3), after which sampling was not carried out with the same level of detail. Note that in many cases a very small number of insects might be found well in advance of any local population growth, even a full year beforehand as shown for the Big Bend and Bunkerville sites in 2009 (Fig. V.3). This is a common occurrence, as there can be as much as a 2-year lag between colonization by adult insects and establishment of a population sufficiently large to cause major defoliation (Dudley and Bean 2011). Regular but less intensive observations were still made continuing into October of both years, and a small number of residual adults apparently representing a weak third generation was present particularly near the ‘defoliation front’ (downstream edge of the defoliation zone). These late insects and the visible defoliation front were present at Hidden Valley, Arizona in 2009 ca. 4 km below Littlefield, and in 2010 the front was at Riverside, Nevada (see map as part of Fig. V.3). These results will provide a consistent record for assessing population patterns over time as establishment expands and deepens in the watershed.

Defoliation followed the typical pattern of rapid desiccation of foliar tissue within ca. 2 weeks of beetle colonization, with numbers in excess of 400 individuals per tree and often as high as 2000 or more (Fig. V.3). After depleting foliage locally, adult beetles fly short distance to find new plants while larvae crawl down defoliated plants in search of nearby plants not yet attacked; most quickly die of starvation (they will not feed on any other plants – Dudley and Kazmer 2007, Moran et al. 2009) or are preyed upon by a wide variety of generalist predators, both arthropods and vertebrates (Bateman et al. 2010, Longland and Dudley 2008). Numerous generalist arthropod predators are found in association with *D. carinulata* on tamarisk plants, including Coccinellidae (ladybird beetles), Reduviidae (assassin bugs), Pentatomidae (stink bugs), Mantidae (preying mantids), Formicidae (ants) and several families of Arachnida (spiders). Data on these predators and other associated invertebrates are archived with Clark Co DCP and provide a baseline for comparing trophic responses over the course of tamarisk decline and ecosystem recovery in the future, particularly as they will form a key part of the future resource base for wildlife inhabiting the mixed tamarisk/native vegetation anticipated to follow from the biocontrol effects.

The original *Diorhabda carinulata* beetles introduced into Utah and Nevada in 2001 were not capable of establishing as far south as the Virgin River owing to their response in Autumn of entering diapause too early in the season (Bean et al. 2007, Dalin et al. 2010). But, we have now documented that there has been natural selection for delaying this response to daylength (Section V-D; Bean et al. 2011), meaning that insects are now capable of establishing further south than the original 38° No. latitude limit, as clearly indicated by the beetles that we collected at the successful establishment sites in Meadow Valley Wash, and the Virgin River watershed which runs from 37.1° to 36.5°N. These studies have been done in collaboration with Dr. Dan Bean, Colorado Dept. of Agriculture, using temperature- and light-controlled incubators at the CDOA facilities in Palisade, CO to test developmental responses by *D. carinulata* collected from southern Nevada and other study areas, as described more fully in Section V-D. It is anticipated that continued evolution of 'improved' developmental response to daylength will enable the beetles to continue its southward dispersal and establishment, at Lake Mead during 2011 or 2012, and perhaps further downstream of Boulder Dam (aka Hoover Dam) into the lower Colorado River reaches in subsequent years. This will also be closely monitored as part of the upcoming Tamarisk Coalition/Walton Family Foundation program to document tamarisk biocontrol progress and ecosystem recovery, and facilitate riparian restoration where beneficial for wildlife enhancement.

Target plant (tamarisk) response to biocontrol

Re-growth of tamarisk foliage at the Virgin River sites occurs within roughly 3 to 5 weeks of beetle defoliation, but often a second generation of beetles is produced that defoliates the plants a second time during the season although the peaks in defoliation extent are often indistinct (Fig. 3, *dark symbols*). Repeat defoliation, within and between years, reduces the capacity of the plant to store metabolites (soluble carbohydrates) within plant tissues, eventually leading to mortality of some plants over the course of 3 or more years (Fig. V.4; Hudgeons et al. 2007, Pattison et al. 2011). However, in some cases mortality occurs much faster, as we documented 15% mortality (3 out of 20 regularly monitoring plants; upub. data) of plants in 2009 at our Cedar Pocket monitoring site (Virgin Gorge, AZ). The final bout of defoliation at this site occurred late in the season, so that plants had no time to compensate for herbivore damage by re-growing foliage which would have enabled some photosynthesis late in the season prior to leaf-drop for the winter. Our co-operator Kevin Hultine (Northern Arizona University) has documented similar rapid tamarisk mortality owing to late defoliation by the leaf beetle at another study site in central Utah (Dolores River; Hultine et al. 2010), indicating that if biocontrol can be targeted at late season periods, the likelihood of rapid mortality may be enhanced.

The goal of biocontrol is not eradication of a target weed, but instead its suppression to a tolerable density so that its negative impacts to ecosystems and biodiversity are ameliorated. This has been observed at our northern Nevada test sites and elsewhere (Pattison et al. 2010, Dudley 2009) where we typically see these massive, 'epidemic' dispersal and defoliation phases settle into an’ endemic’ mode as tamarisk live biomass is diminished and also as various predators respond to this new food resource (the beetles) to increase in local abundance (Longland and Dudley 2008, Dudley and Bean 2011).The data we collected in Clark County in 2009 and 2010, along with our earlier sampling starting in 2006 soon after introduction of *D. carinulata* took place in Utah, so far follow a similar trajectory. Beetle numbers along the Santa Clara and Virgin Rivers in Washington County, Utah, have declined since introduction as plant biomass and predators have both responded to their presence, and tamarisk plants in the Utah reaches have greatly diminished vegetative cover while native plants are abundant (qualitative data not presented here, and vegetation management, e.g. weed control and willow restoration actions, in the St. George area make quantitative assessment infeasible).

Within Arizona and Nevada, the regular monitoring data showing live-foliar cover of tamarisk during the field seasons of 2009 and 2010, at least up to August of 2010 when funding for the sampling crews was depleted (Fig. V.3). Along with vegetation surveys for the MSHCP (Section I, this report) and those that we conducted as a separate project (P. Shafroth and S. Ostoja, USGS unpub. data), this information will provide a detailed and comprehensive baseline dataset for monitoring long-term responses to tamarisk biocontrol, both of target weeds and other native and non-native plant species responses to tamarisk dieback. That program will continue over a minimum 5-year period as part of the UCSB/Walton Family Foundation/Tamarisk Coalition program to facilitate riparian ecosystem recovery and restoration for Covered species, particularly the SW willow flycatcher.

At this time it is too early to document significant dieback or sustained reduction in vegetative cover of tamarisk except for the small number of plants killed, surprisingly, at the Virgin Gorge (Cedar Pocket) monitoring station, so only the short-term defoliation reduction in cover, and subsequent replacement by re-growth, is evidenced by the baseline data (Fig V.5). This is too short a period for any recovery responses by associated vegetation, which will be documented during future monitoring using the same monitoring locations, although it is important for wildlife species that may be nesting at the time of defoliation. The timing of defoliation in the lower reaches of the Virgin River (Arizona and Nevada) generally did not coincide with the nesting period of most birds, including the willow flycatcher (Appendix I, this report) despite its generally late nesting behavior (McLoed and Koronkeiwics 2010). That may not be the case during 2011 and subsequent years as beetles will not have to disperse and oviposit (lay eggs) prior to developing sufficient population size to cause defoliation.

We anticipate that within Clark County the very large volume of tamarisk biomass present (estimated to cover as many as 5,000 acres or more) will result in a massive *Diorhabda* population growth rate, similar to what we have seen in other heavily infested systems, and the population sustained over the course of roughly 2 years. Then it will likely decline as target plants die back and other factors mitigate the size of the beetle population, and the plant monitoring protocol is in place to document plant responses during that period.

It should be noted that the majority of the tamarisk present in the Virgin watershed is comprised of the central Asian species and their hybrids (*Tamarix ramossisima, T. chinensis*; Gaskin and Schall 2002); however, a Mediterranean species, *Tamarix parviflora*, makes up a substantial minority of the tamarisk vegetation in the Virgin River in all three states (Dudley, unpub. data). The composition of tamarisk vegetation from the full extent of the lower Virgin River is mapped in collaboration with USGS (P. Shafroth and S. Ostoja, unpub. data) with funding from the USGS Invasive Species Program, so those data are not included in this reporting to Clark County. This species from the more temperate climate conditions of the Mediterranean region flowers earlier than the central Asian forms (Dudley et al. 2011), and in its geographical origin does not coincide with the *D. carinulata* beetle that is present in the Virgin watershed. It has been shown that this beetle species does not recognize *T. parviflora* as a suitable host plant for oviposition in the field, despite feeding by beetles on this plant in the laboratory (Dalin et al. 2010, Dudley et al. 2011). We observed some limited use of *T. parviflora* by beetles during 2009 and 2010 when beetle population sizes were extremely high, primarily because larvae that retreated from defoliated *T. ramossisima/T. chinensis* plants do not distinguish between plant types (Moran et al. 2008, Dalin et al. 2010). Starving larvae encountered these *T. parviflora* trees and fed on them; however, later in the season when the succeeding generation of beetles was lower in abundance, the *T. parviflora* plants were largely untouched by these herbivores (Dudley, unpub. data). The implication of this selectivity is that, if defoliation does have negative impacts on potential breeding site of southwestern willow flycatchers in areas with no native plant options, *T. parviflora* will retain the vast majority of its foliar cover so is likely to remain suitable for nesting.

Figure V.1. *Diorhabda* distribution in Colorado River Basin (a) and the Virgin River (b) by August 2010.

a.

b.

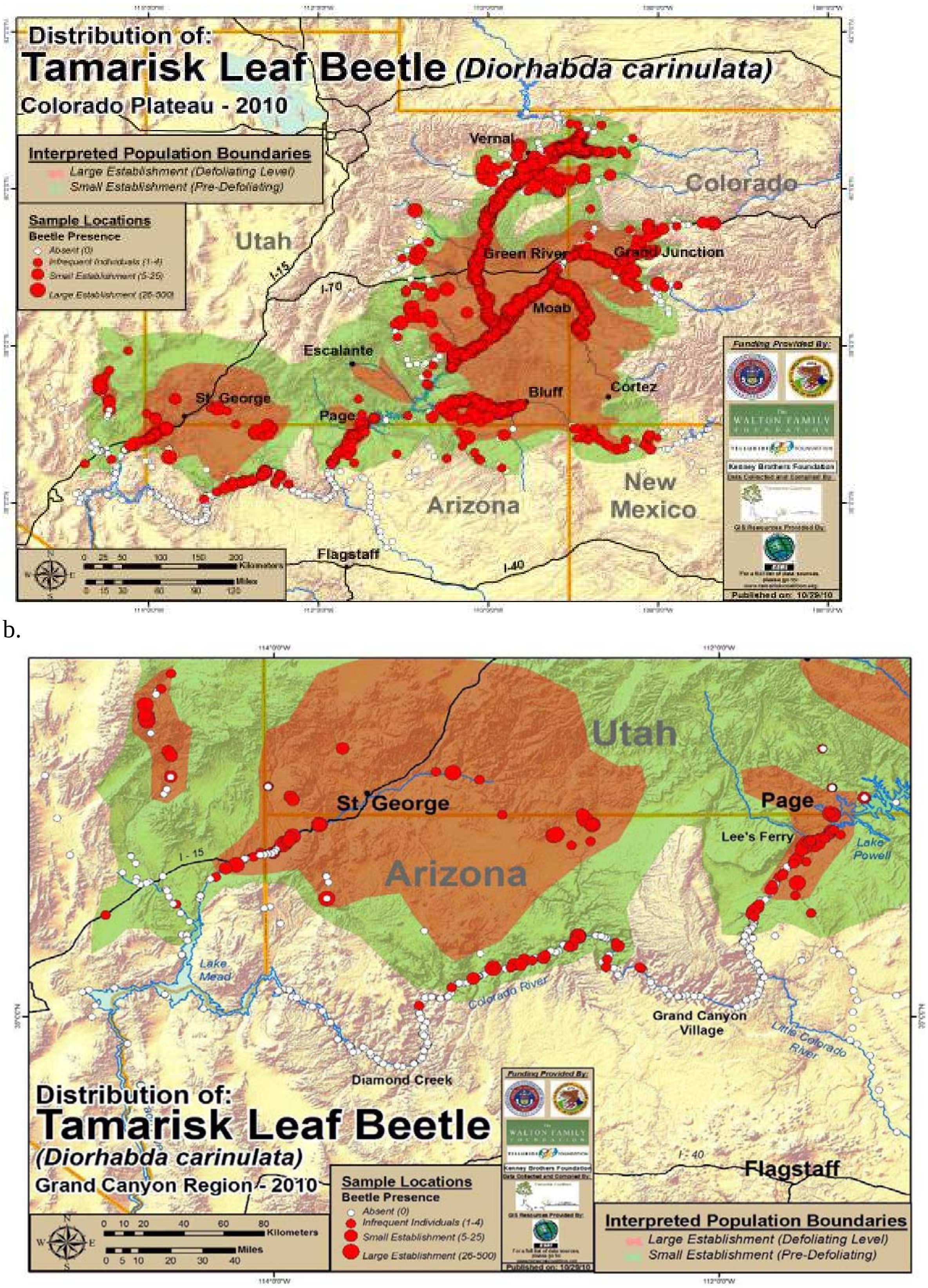
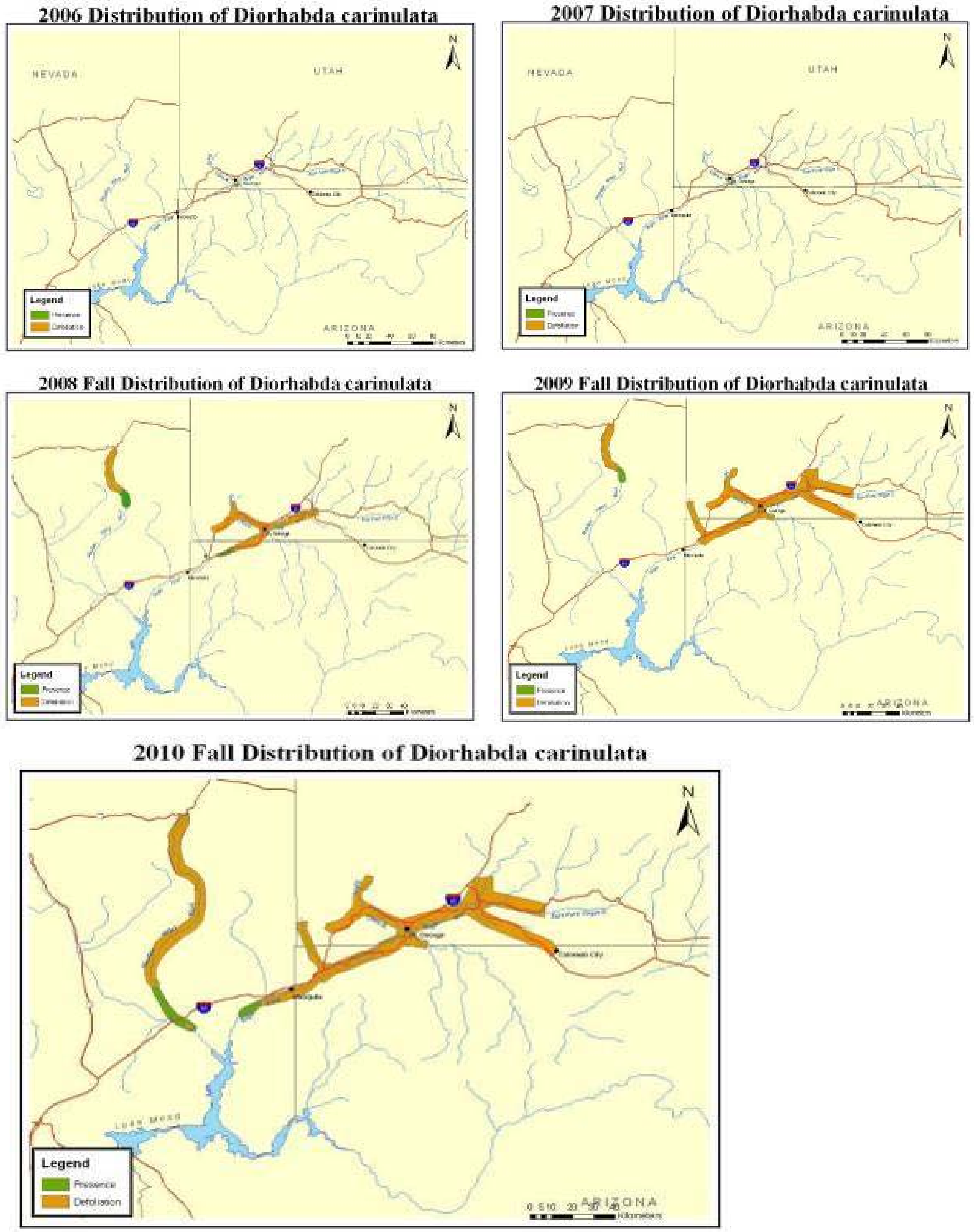


Figure V.2. Expanding distribution of *D. carinulata* in the Virgin River watershed since introduction.



2006 Distribution of Diorhabda carinulata

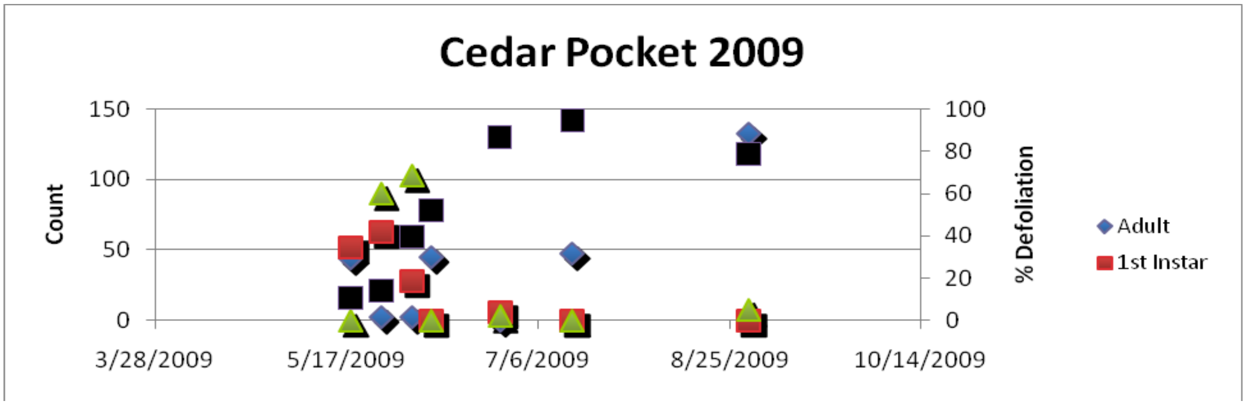
2007 Distribution of Diorhabda carinulata

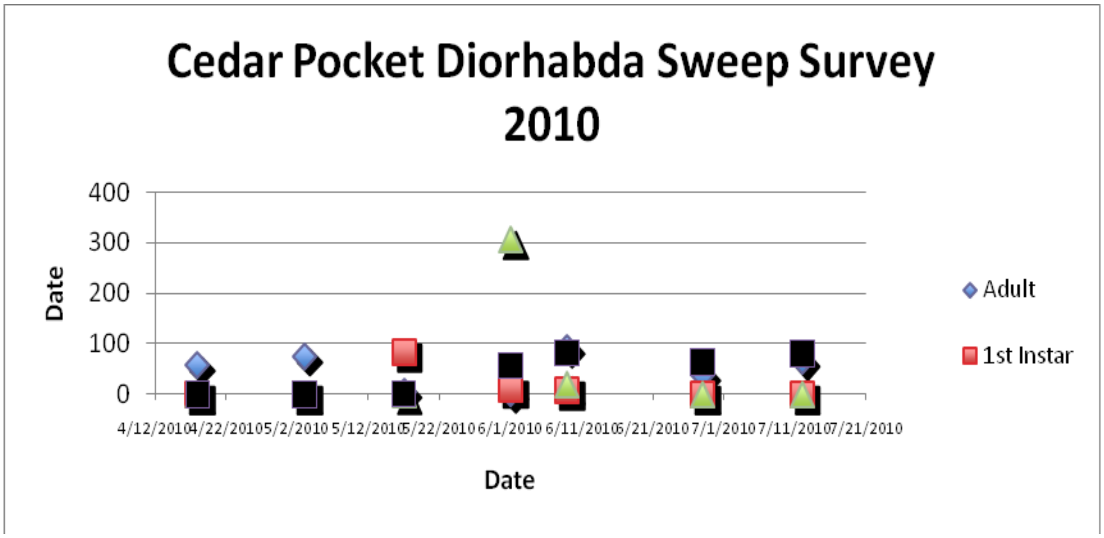
2008 Fall Distribution of Diorhabda carinulata

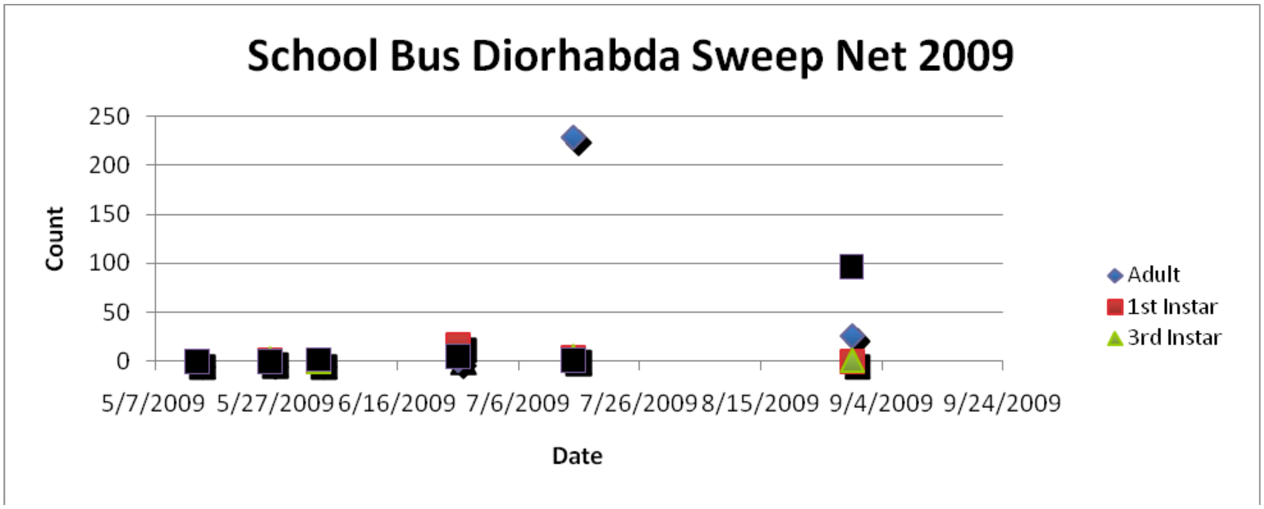
2009 Fall Distribution of Diorhabda carinulata

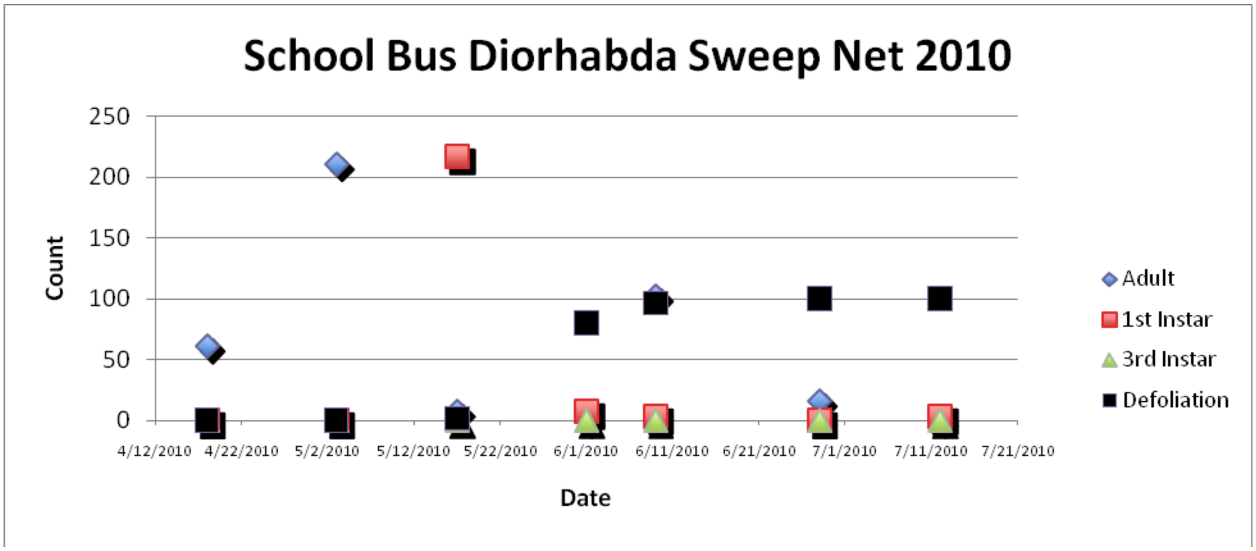
2010 Fall Distribution of Diorhabda carinulata

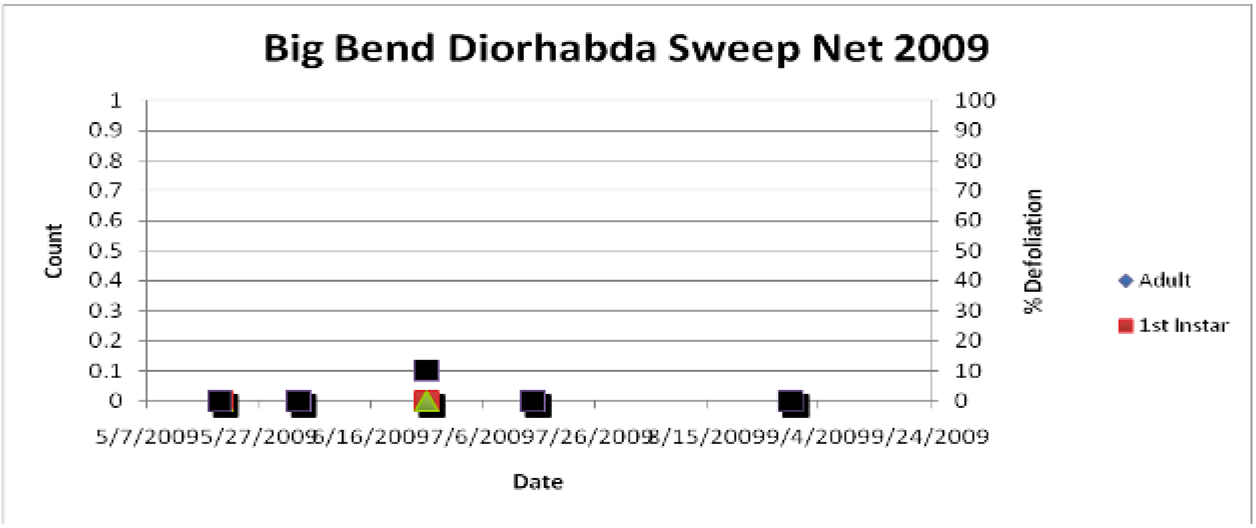
Figures V.3. Sweep net surveys of *Diorhabda* abundances and tamarisk defoliation at monitoring sites in the lower Virgin River watershed. No chart is given for Mormon Mesa sites, as *Diorhabda* was not present in the lower river segments prior to 2011. A map following graphs shows site locations.

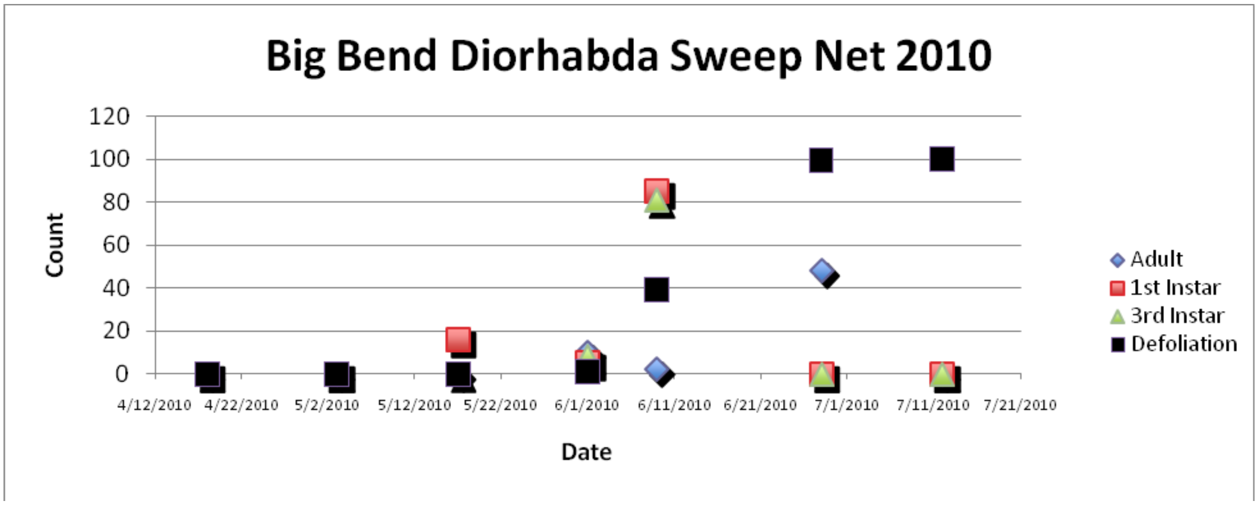


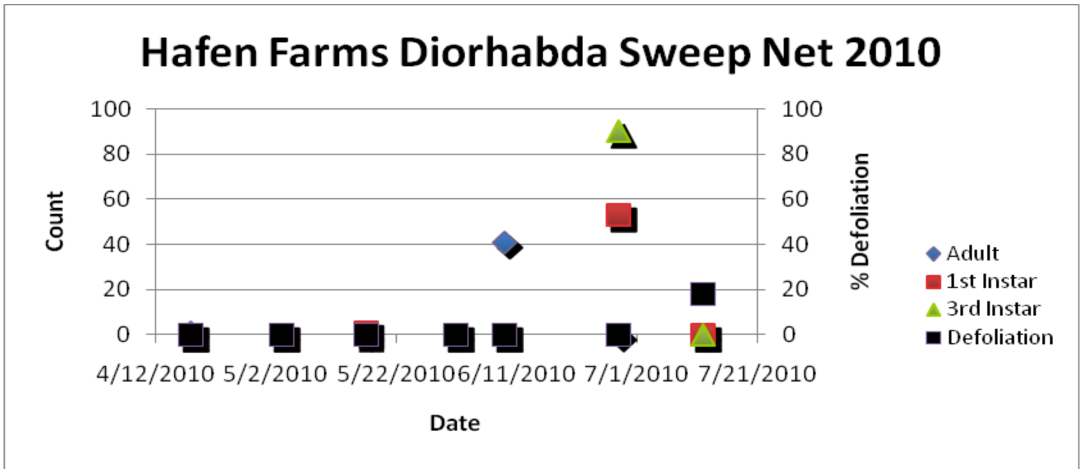


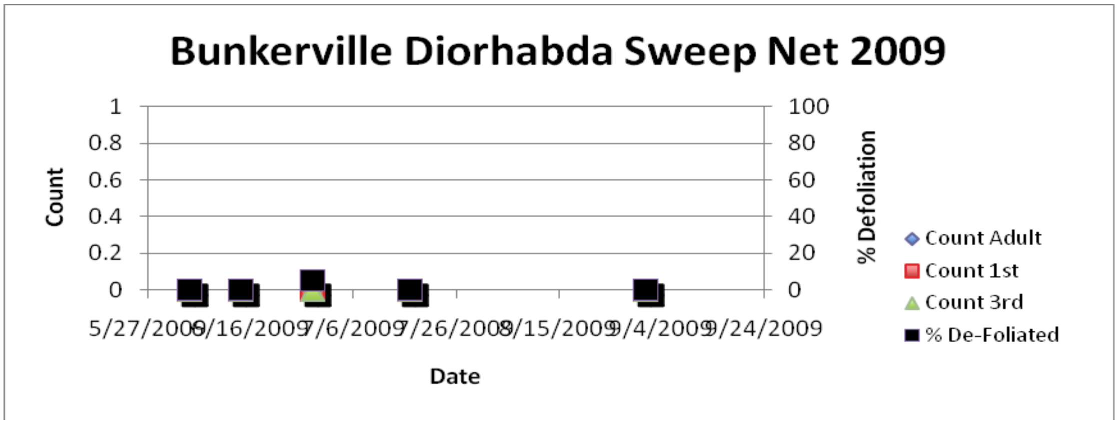


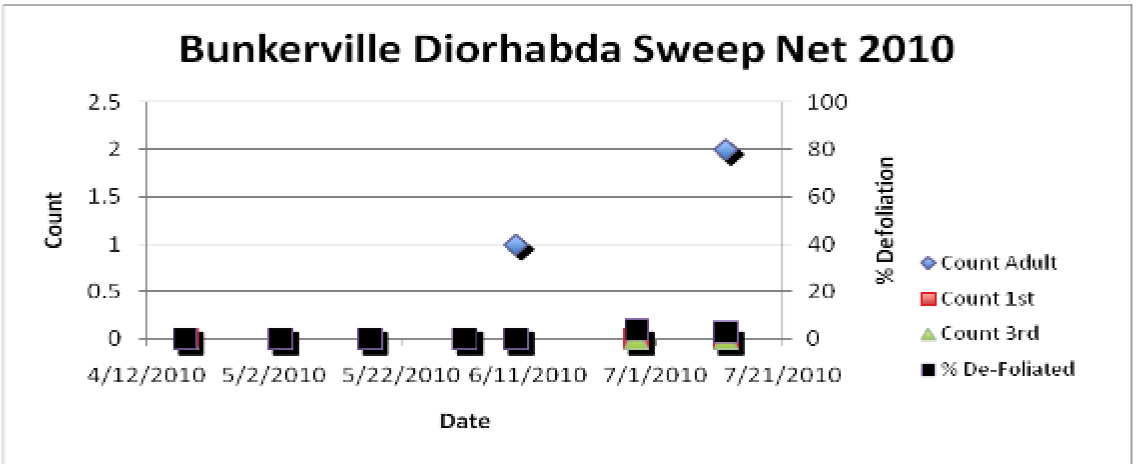

School Bus Diorhabda Sweep Net 2010
Count
250
200
150
100
50
0
4/12/2010
4/22/2010
5/2/2010
5/12/2010
5/22/2010
6/1/2010
6/11/2010
6/21/2010
7/1/2010
7/11/2010
7/21/2010
Date
◆ Adult
■ 1st Instar
▲ 3rd Instar
■ Defoliation

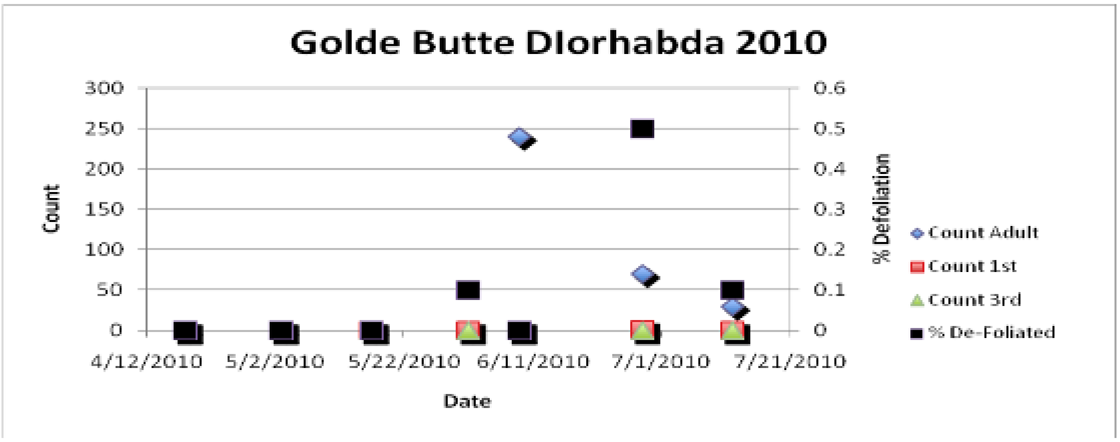

Big Bend Diorhabda Sweep Net 2009
Count
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100
90
80
70
60
50
40
30
20
10
0
% Defoliation
5/7/2009
5/27/2009
6/16/2009
7/6/2009
7/26/2009
8/15/2009
9/4/2009
9/24/2009
Date
◆ Adult
■ 1st Instar


Big Bend Diorhabda Sweep Net 2010
Count
120
100
80
60
40
20
0
4/12/2010
4/22/2010
5/2/2010
5/12/2010
5/22/2010
6/1/2010
6/11/2010
6/21/2010
7/1/2010
7/11/2010
7/21/2010
Date
◆ Adult
■ 1st Instar
▲ 3rd Instar
■ Defoliation


Hafen Farms Diorhabda Sweep Net 2010
Count
% Defoliation
100
80
60
40
20
0
4/12/2010
5/2/2010
5/22/2010
6/11/2010
7/1/2010
7/21/2010
Date
Adult
1st Instar
3rd Instar
Defoliation


Bunkerville Diorhabda Sweep Net 2009
Count
% Defoliation
1
0.8
0.6
0.4
0.2
0
100
80
60
40
20
0
5/27/2009
6/16/2009
7/6/2009
7/26/2009
8/15/2009
9/4/2009
9/24/2009
Date
Count Adult
Count 1st
Count 3rd
% De-Foliated


Bunkerville Diorhabda Sweep Net 2010
Count
% Defoliation
2.5
2
1.5
1
0.5
0
100
80
60
40
20
0
4/12/2010
5/2/2010
5/22/2010
6/11/2010
7/1/2010
7/21/2010
Date
Count Adult
Count 1st
Count 3rd
% De-Foliated


Golde Butte DIorhabda 2010
Count
% Defoliation
300
250
200
150
100
50
0
0.6
0.5
0.4
0.3
0.2
0.1
0
4/12/2010
5/2/2010
5/22/2010
6/11/2010
7/1/2010
7/21/2010
Date
Count Adult
Count 1st
Count 3rd
% De-Foliated

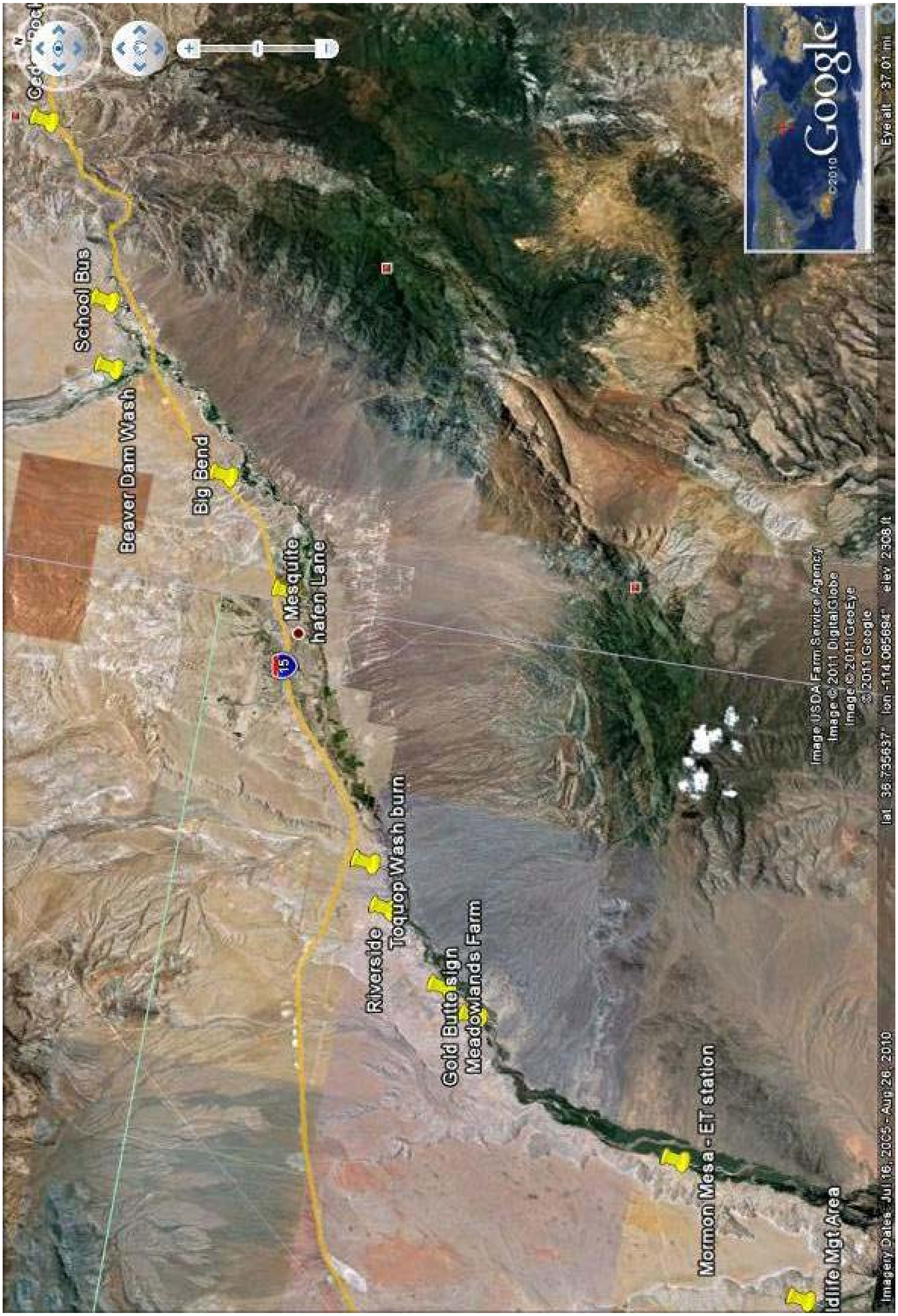
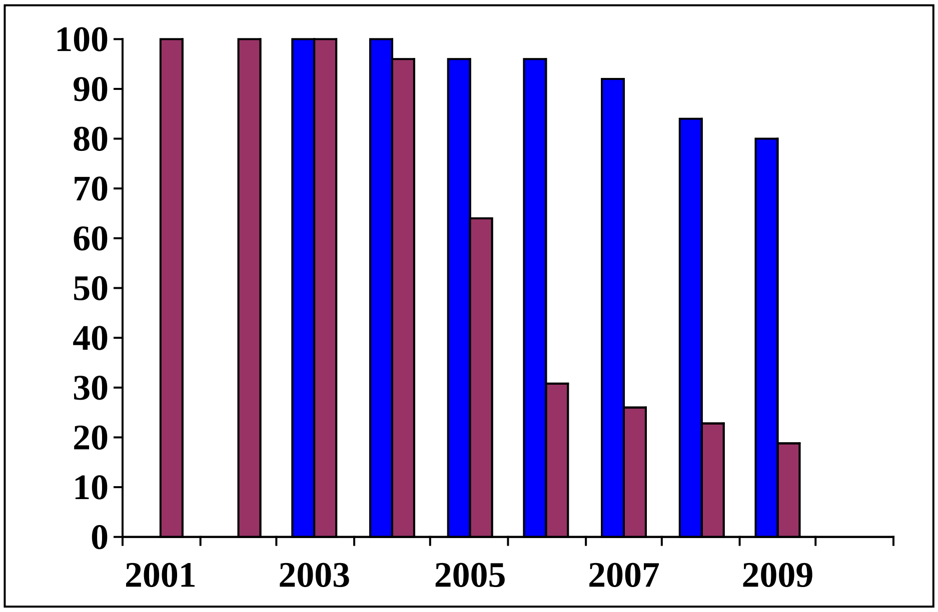

School Bus
Beaver Dam Wash
Big Bend
Mesquite
hafen Lane
15
Toquop Wash burn
Riverside
Gold Butte sign
Meadowlands Farm
Mormon Mesa - ET station
ldlife Mgt Area
Image USDA Farm Service Agency
Image © 2011 DigitalGlobe
Image © 2011 GeoEye
© 2011 Google
©2010 Google
Imagery Dates: Jul 16, 2005 - Aug 26, 2010
lat 36.735637° lon -114.065694° elev 2308 ft
Eye alt 37.01 mi

Figure V.4. Mortality of monitored *T. ramosissima* trees (min. N = 36 per location) at the central Nevada biocontrol research site (Humboldt Basin) resulting from repeated defoliation by *D. carinulata*. Survivorship is indicated for two locations, the release point in purple, which received intense repeat defoliation starting in 2002 following release in 2001, and in blue the monitoring station 2 km away from the release point where biocontrol insects established in 2004, and defoliation occurred repeatedly but with lesser intensity. These data would correspond to tamarisk mortality from biocontrol in Clark County in 2013 or 2014.



Section V-B. Other Elements related to Tamarisk Biocontrol

Biocontrol and Plant Responses Co-operative studies with Drs. Kumud Acharya and Ben Conrad (Desert Research Institute) are assessing tamarisk ecophysiological status across a chronosequence of plants affected for 1, 2, 3 or 4 years to characterize how herbivory by *Diorhabda* stresses host plants, ultimately leading to mortality. Current plans are also to determine how herbivory influences tamarisk ecophysiological condition at the Virgin River and what triggers eventual host plant mortality. A larger-scale proposed program will evaluate changes in the Colorado River Basin riparian communities as they relate to Climate Change and Invasive Species, with the focus on using the Virgin River as the research watershed for evaluating these issues.

Other Specialist Herbivores of Tamarisk We will also continue investigations of the newly discovered specialist tamarisk weevil, *Coniatus* sp., discovered at Las Vegas Wash this year (probably *C. splendidulus*; a closely related species had previously been investigated as a potential biocontrol agent) to evaluate its potential to suppress tamarisk. *Coniatus* has subsequently been detected on the Virgin River in Utah and Nevada, and at the Bill Williams River in 2010.

Monitoring programs routinely incorporate the effects of an unintentionally introduced leafhopper, *Opsius stactogalus*, which often causes extensive yellowing and reduced vigor in tamarisk plants, particularly those stressed by factors such as drought or herbivory by *Diorhabda*.

Biocontrol and Fire Management Defoliation during the growing season can slightly increase wildfire risk during the growing season (see Section III-B), but biocontrol may also reduce the risk of wildfire if it results in plants dropping litter (particularly if windy conditions ensue) rather than being retained on the plant. Interestingly, there were no major wildfires during 2010 while plants along the Virgin River and other tributary systems were in defoliated condition, but this is more likely due to happenstance than resistance to burning by leafless vegetation. The elements of this project that concern wildfire strongly support the hypothesis that defoliation does reduce fire risk over time (Section III-C, Drus et al. 2011, Brooks et al. 2008), a fact that is being taken into account by fire managers in Clark County and the wider region (N. Caplette & S. McEldery, BLM, pers. communication). Alternatively, we are in discussion with fuel managers from BLM (Caplette & McEldery – Las Vegas office, Susan Williams – Ely office) and NPS (Deuser) about the timing and circumstances suited to using fire as a prescription tool to reduce flammable tamarisk biomass and, equally important, to enhance target weed mortality owing to the combined effects of biocontrol reduction of plant vigor and metabolite storage for re-growth, and direct fire damage to tissues (Drus et al. in press; Brooks et al. 2009).

Section V-C. Temperature Effects on Life History Traits of *Diorhabda carinulata*

Background

Day length is the primary factor causing diapause induction of *Diorhabda* spp. beetles, with specific responses depending on ecotypes (Dalin et al. 2010) and temperature (Bean et al. 2007). Under constant temperature, critical day length shortens as temperature increases, and it is not clear for the population of *D. carinulata* established and expanding at the Virgin River how the temperatures that are routinely > 40° C (105° F) may affect the induction of diapause in this ecosystem. Other insects have different reactions and in several species, diapause cannot be induced at any photoperiod above a particular temperature (Danks 1987). However, the most important ecophysiological variable affecting the performance of the beetles is body temperature (Angilletta et al. 2002). Ectotherms respond to heterogeneity of the thermal environment in several ways. For instance, developmental date of *Liposcelis paeta* decreased as temperature increased from the constant 22.5 °C to 37.5°C, but survivability was high at medium temperature (Wang et al. 2009). Similar trends were observed with *Cionus latefasciatus* (Xu et al. 2009) and leaf beetles.

Current research has shown that *D. carinulata* develops faster when temperature increases under the conditions between 24 °C and 31 °C average temperatures (Lewis et al. 2003). Herrera et al (2005) examined temperature dependent developmental rates under constant temperatures between 15 °C and 40 °C and concluded that too low or high temperatures increase development time. They also observed that beetles are sensitive to extreme temperatures (15 °C and 40 °C) and have high mortality. All of these studies were done at constant controlled temperatures in the lab, which is not representative of what the beetles experience in their natural ecosystem. Therefore it is important to study the physiology of beetles under fluctuating temperatures so that a better understanding of the life history characteristics can be determined.

Methods

Experimental Animals *Diorhabda carinulata* used in this study originated in Chilik, Kazakhstan (DeLoach et al. 2003) and were recollected from the biological control colonization zone along the Virgin River from St. George, UT to Littlefield, AZ. Beetles were collected in June, 2009 for establishment of a laboratory colony and included both larvae and adults. The stock cultures were kept at room temperature (~ 23 °C) with a 16:8 (Length:Day) photoperiod. Larvae and adults were kept separate in plastic containers with screen lids and supplied with fresh cuttings of *T. ramosissima* which were made into bouquets with cut ends in vials of water to preserve foliage quality. Plastic containers were cleaned and bouquets were replaced every day.

Experimental Design Experiments were conducted in the Ecological Engineering Laboratory, Desert Research Institute, Las Vegas, Nevada. Experiments were conducted under three different temperature conditions: (i) room temperature (23 °C); (ii) constant high (31°C); and (iii) variable high from 25-40°C (with average 31°C). Constant and variable high temperature experiments were conducted using controlled environment chambers (Model A1000I, Conviron, North Dakota, USA). Temperatures for variable high treatment were varied from 25 ºC (lowest) to 40 ºC (highest) within 24 hours. The temperature routine included 8 hours of 25 ºC without light followed by 4 hours of 30 ºC, 2 hours of 35 ºC, 4 hours of 40 ºC and 2 hours of 35 ºC, and 4 hours of 30 ºC all under lighted conditions (Figure V.6). Temperature step decrease and increase was set at the rate of 0.5 °C /min. The photoperiod setting for all three treatments was 16:8 (L:D) to ensure none of beetles went into diapause during experiments as reported in Bean et al. (2007).

Experiments were initiated in September, 2009. The weights of beetles at the beginning of each larval stage (immediately after molting) and development time were recorded. These measurements were then used to estimate growth rates at each larval stage. Eggs produced by adult beetles were also counted for approximately a week to monitor their reproduction under the three treatment conditions. Each treatment initially had fifty eggs that were placed in 669 ml plastic containers that contained foliage in small eppendorf vials. The container was then kept at experimental temperature and light. The first instars were moved to 4 L plastic containers within 24 hours of their hatching and fresh eppendorf bouquets were supplied daily. When third instars ceased feeding and were ready to pupate, they were moved to containers with a 2 to 3 cm deep layer of medium grained sand for pupation. Emerging adults were immediately provided with foliage and transferred into 4 L plastic containers with fresh bouquets with cut ends in vials of water. There were three replicates for each treatment. During the experiments, all plastic containers were cleaned daily and fresh Tamarisk bouquets were supplied while dead animals were counted and removed. Tamarisk foliages were collected from the Wetlands Park, Las Vegas, Nevada every two weeks and stored in the refrigerator.

Randomly selected five instars from each container were individually weighed using a Denver Instrument balance (model PI-225D, New York, USA). Growth rate (μ: in units of d^{-1}) for each larval stage was, then, calculated according to the following formula:

$$\mu = \ln(\text{final weight}/\text{initial weight})/\text{number of days} \qquad \text{Eq.(1)}$$

Statistical Analysis One-way analysis of variance (ANOVA) was used to study the effect of temperature on developmental rate, body weight, growth rate, reproduction, and mortality. In particular, Tukey's HSD test was carried out to compare between treatments using JMP software (SAS Institute Inc., Cary, NC) and p values < 0.05 (95 % confidence level) were considered significant.

Results and Interpretations

Results The effect of temperature on beetle body weight is shown in Figure V.7. Adult beetles grown in the room temperature treatment had a significantly higher body weight than those in both the constant high and variable high treatments. Additionally, the constant high temperature treatments produced adult beetles with greater body weight than those in the variable high treatment. Finally, the room temperature and constant high treatments produced pupa with significantly higher body weight than those in the variable temperature treatment. The average weight of larvae a day before pupating in room, constant high and variable high treatments were approximately 13, 12 and 10 mg and a day after emerging as adult beetles were about 8, 6 and 5 mg, respectively.

The temperature effects on developmental time are shown in Figure V.8 and Table V.1. Beetles in the constant high temperature took the shortest time (25.4 days) to reach adult eclosion from egg while beetles in the room temperature took the longest (61.2 days). Number of days taken from the birth to maturity under variable high temperature fell between that of the constant high and room temperature treatments at 34.1 days.

Constant high temperatures yielded the highest growth rates among all the stages (between 0.17 and 0.39) while the slowest growth rates were observed in the room temperature (between 0.14 and 0.16) (Figure V.9). The beetles in the variable high temperate treatment showed slightly higher growth rates than those in the room temperature treatments (between 0.18 and 0.21).

Beetle reproductive fitness (number of eggs per female) for the different temperature regimes are shown in Figure V.10. The room temperature treatment had the lowest average reproduction (3 eggs per day) whereas the constant high had the highest (8 eggs per day). Reproduction in the variable high temperature fell in between at 5 eggs per day. Furthermore, pre-oviposition period was also affected by the temperature. As shown in Table V.1, beetles in the room and variable high conditions took much longer to start producing eggs (≈ 9 days) while those in the constant high took only 3.7 days. Finally, temperature also had a significant effect on survivability. The variable high temperature condition had the highest mortality rates (Fig. V.11). The animals in this treatment were most vulnerable at the 1st instar with more than 45% mortality, and nearly 25 % died during pupation. The constant high temperature treatment had the lowest mortality (< 15 %).

Interpretation Experimental results showed that temperature affected weights of *D. carinulata*. The average weight of leaf beetles was reduced with increasing temperature. Similar to the leaf beetles, *Manduca sexta* also shows decreases in weight with increasing temperature (Davidowitz et al. 2004). Also, Atkinson and Sibly (1997) reviewed that larger body size was observed at cooler temperatures in 83% of the 109 studies of ectotherms. However it has been shown that not only temperature effects body size of insects, but also exposure time. For example, Lee and Roh (2010) showed that there were no significant differences in caterpillar body size based on temperature in their experiment. They explain that this may have been an effect resulting from only a brief exposure time at higher temperatures during their larval feeding stage. For our study, varied temperature treatments remained throughout the entire experiment.

Developmental time showed clear differences based on the temperature conditions. When temperature was increased from the room to the constant high, developmental time was reduced significantly. Decrease in developmental time with increase in temperature has been reported by many researchers; Lewis et al. (2003) and Herrera et al. (2005) for leaf beetles, Xu et al. (2009) for *Cionus latefasciatus*, Wang et al. (2009) for *Liposcelis paeta* and Gotoh et al. (2010) for *Tetranychus evansi*. Our study also suggested that the effect of temperature on developmental time differs between a variable high and a constant high temperatures despite both having the same average temperature. The variable high temperature increased developmental time compared to the constant high temperature in our experiment. Our results of the effects of temperature on developmental time in the tamarisk leaf beetle suggest that there may be an optimal temperature range beyond which developmental time is significantly impacted. This was also shown in results by Zhou et al. (2010) on the leaf beetle *Ophraella communa*. In their study on the effects of temperature on developmental time, the optimal range for development for O. *communa* was between 25-28 °C. At temperatures much beyond this range developmental time either greatly increased or there was complete mortality.

The beetles in our experiments generally had higher growth rates in high temperature conditions (both constant and variable high). Lee and Roh (2010) reported that growth rate of *Spodoptera exigua* increased as temperature increased. They also pointed out that higher growth rate was because of their shorter developmental time at higher temperatures rather than weight differences. Additionally, Atkinson and Sibly (1997) suggested that average individual growth rates decreased with decreasing temperatures while body size increased at cooler temperatures. This was also the case with *Manduca sexta* (Davidowitz et al. 2004) and for *D. carinulata* in our study. In our experiments, both weight and development time appears to contribute to changes in growth rates. Furthermore, there were differences between growth rates in the constant high and variable high temperature treatments. The variable high treatment had a slower growth rate than the constant high treatment, again suggesting an optimum temperature for development. Herrera et al. (2005) suggest that this optiumum temperature lies between 30-35 °C. Since our constant temperature lies within this range, and our variable temperature extends beyond, it is not surprising that differences in growth rates between these treatments were observed.

The variable high temperature condition showed a significantly higher mortality compared to the constant high temperature. This might be contributed by the upper end of the temperature ranges in our experiment. As reported in Herrera et al. (2005), all the eggs and pupa did not survive and more than 60 % larvae died at a constant of 40 °C temperature. In our experiment, four hours of 40 °C exposure did not damage all the eggs or pupa but the damage was significant. 1st instar larvae and pupa had > 45 % and 25 % mortality respectively. Previous research suggest that insect thermal tolerance is highly variable and largely depends on the age of the organism and generally supports the notion that high temperature limits decline with age and into more advanced life stages (Bowler and Terblanche, 2008). Gotoh et al. (2010) also showed that the different strains of the tomato red spider mite react differently under similar temperatures. Herrera et al. (2005) showed that *D. carinulata* (called *D. elongata* in that report) seemed to experience lower mortality as they grew from 1st to 3rd instars at lower temperatures while 3rd instars were more sensitive to higher temperatures.

Our results showed that temperature fluctuation in early stages may be quite harmful to growth of *D. carinulata*. Interestingly, diurnal temperature fluctuation did not cause a significant damage to egg hatchings in our study unlike in the constant high temperature of 40 °C in Herrerra et al. (2005). Davison (1969) reported that the heat death points (temperature which causes 50 % of test animals to die under 40 minute exposure) can vary depending on different stages in life. *Calliphora erythrocephala* showed increase in heat tolerance as they grow older at larval stage and decreased tolerance while pupating. However, the most sensitive stage was during egg incubation. Renault et al. (2004) reported that tropical beetles survive longer at 0°C constant exposure if they are initially kept at a temperature of 15°C when compared to an initial exposure of 30°C. They also showed that exposure to daily two hours of higher temperature up to 30°C helped them survive longer under colder temperatures compared to without exposure. Gradual change of environment (0°C environment from 15 °C instead of 30°C) may represent physiological constraint of these beetles to survive rapid large temperature swings.

These experimental results clearly showed increase in mortalities under variable high temperature compared to the constant temperature conditions despite the same average temperature during early stages of development (1st instar-3rd instar). Furthermore, the variable high temperature had increased mortality in adults when compared to the room temperature treatment, while the constant high treatment did not show significant differences in mortality between both the room and variable temperature at the adult life stage.

Pre-oviposition period and daily egg production were also affected by temperature. Previous studies showed that total egg production is higher at moderate temperatures for two species of whitefly (Greenberg et al. 2000), *Liposcelis paeta* (Wang et al. 2009) and *Tetranychus evansi* (Gotoh et al. 2010) over lower or higher temperatures. On the other hand, Wang et al. (2009) suggested that higher than normal temperature affected olive fruit fly reproduction negatively. When the maximum temperature was increased from 23.9 °C to 37.8 °C keeping the lowest temperature same, number of eggs produced per female was reduced. This is similar to our variable high condition, where the number of eggs reduced. On top of the higher reproduction number, beetles in the constant high condition started producing eggs much earlier than the other two conditions. It is possible that the fecundity differences observed between the constant high and variable high treatments are a result of a heat shock protein up-regulation. Although we did not measure this parameter, Dahlhoff et al. (2008) found that when the willow beetle (*Chrysomela aeneicollis)* was acclimated for 4 hours at an elevated temperature the heat shock protein Hsp 70 was positively correlated with fecundity. Therefore it could be that in our constant high treatment beetles are under constant heat stress which could result in a greater upregulation of Hsp 70 than the beetles in the variable high treatment.

Further analysis and experimentation would be needed to explore this hypothesis. In addition to temperature effects on number of eggs produced, pre-oviposition was also impacted. When temperature was increased from room to constant high, pre-oviposition period was reduced but when beetles were kept in the variable high, pre-oviposition period increased. Greenberg et al. (2000) reported that two different species of whitefly showed little difference in pre-oviposition period. *T. vaporariorum* had the shortest pre-oviposition at milder temperature condition while *B. argentifolii* reduced pre-oviposition period almost linearly as temperature increased. Leaf beetles appear to have similar trends as *T. vaporariorum*.

One of the important environmental conditions for leaf beetles is humidity. It has been found that survivorship of beetles decrease when humidity increases. If the relative humidity in experimental growth chambers is higher than 50%, it is necessary to lower humidity. Our three experimental conditions, room, constant high and variable high temperatures had less than 25% relative humidity and there was no noticeable frass on tamarisk foliage. Therefore, we do not think humidity affected our treatments in anyway. We did notice that higher temperatures (constant and variable high treatments) tended to dry out tamarisk's foliage by the end of 24 hour cycle. Similarly, quality of tamarisks foliage is critical. As DeLoach et al. (2003) reported that quality of foliage affects feeding and development of larvae, especially neonate larvae, there is a possibility that tamarisk might affect the experiment outcomes. Young and fresh tamarisks were collected for our experiments and all three treatments received foliage from the same collection, therefore, we rule out the possibility of any food quality effect variation. In this study, we did not investigate the feeding habits of *D. carinulata*, but based on differences in size under various temperatures, feeding rate might change depending on its ambient environmental conditions.

These results have potential implications for the tamarisk beetle biocontrol program in the southwestern U.S. where *D. carinulata* continues to spread southward in the Virgin and Colorado Rivers (Dudley and Bean 2011). The beetles will experience higher temperature as they disperse and colonize further south, and may routinely experience temperature equal to or greater than 40°C for several hours, similar to our experimental conditions. This factor may be moderated, however, because within the canopy temperatures are generally cooler than outside the canopy. Kotzen (2003) reported that differences between average ground temperature under tree canopy and outside the canopy was more than 5°C and it could be 10°C depending on tree type. Our experimental results showed short exposure of 40°C did not cause complete mortality in the beetles, therefore it is possible that the beetles can move further south to expand their habitat, simply based on their thermal tolerance. Moreover, latitudinal variation in the absolute maximum, minimum temperatures reported in Gaston (1999) showed that minimum temperatures increase as latitude decreases but maximum temperatures shows only slight variation so the resulting temperature range is not extreme. Therefore, it might be easier for beetles to adapt to the lower latitude environment because leaf beetles tend to prefer warmer environments. They may be limited by developmental constraints as day length decreases in southern latitudes, and that was one of the reasons beetles failed to establish at some of the original field sites (DeLoach et al. 2004). However, field experiments by Dalin et al. (2010) suggest that beetles may have evolved new critical daylengths for diapause development in North America, presumably shorter than the original population which indicates that beetles are likely to have the capacity to establish further south and expand their distributional range as explained in Section V-D of this report.

Conclusions Experimental results showed that temperature affects *D. carinulata* developmental time, mortalities and reproduction. One of the significant observations from these experiments was that variable temperature affects not only development time but also size of beetles. For instance, the variable high condition had a longer developmental time than the constant high condition, but shorter developmental time than the room temperature treatment. Furthermore, the variable high adult weights where significantly lower than both the room and constant high treatments. Our results also suggested that extreme temperature negatively affects growth rates and increase mortality. When beetles were kept in fluctuating temperatures between 25 ºC and 40 ºC within 24 hours, they took more time to grow, gained less weight and higher mortality compared to those kept in at constant 31 °C temperature.

Table V.1: Number of days (+/- 1 SE) for each stage

	Egg	1st Instar	2nd Instar	3rd Instar	Pupating	Pre-oviposition
Room	10.3 ± 0.20 A	8.2 ± 0.92 A	8.2 ± 0.34 A	10.9 ± 0.23 A	14.7 ± 0.39 A	9.0 ± 1.00 A
Constant high	5.0 ± 0.01 C	3.2 ± 0.16 B	3.4 ± 0.25 C	5.4 ± 0.12 C	8.3 ± 0.10 B	3.7 ± 0.33 B
Variable high	6.5 ± 0.21 B	5.5 ± 0.27 B	5.2 ± 0.43 B	8.6 ± 0.77 B	8.4 ± 0.31 B	8.7 ± 1.45 A

Capital letters (A, B, C) represent results of Tukey’s HSD test result within same column.

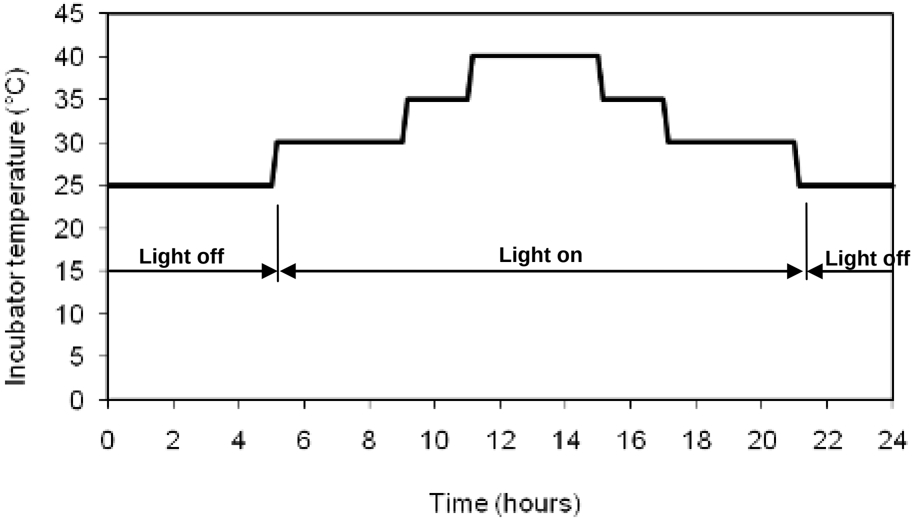


Figure V.6. Temperature and light cycle for variable high conditions

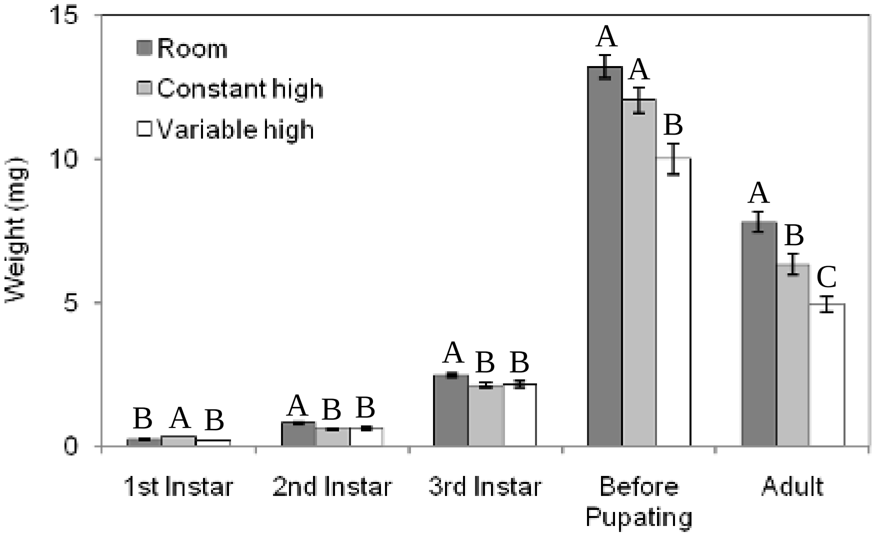


Figure V.7. Average weight of individual insect at beginning of each stage. Capital letters (A, B, C) in the figure represent results of Tukey's HSD test result; error bars are one SE.

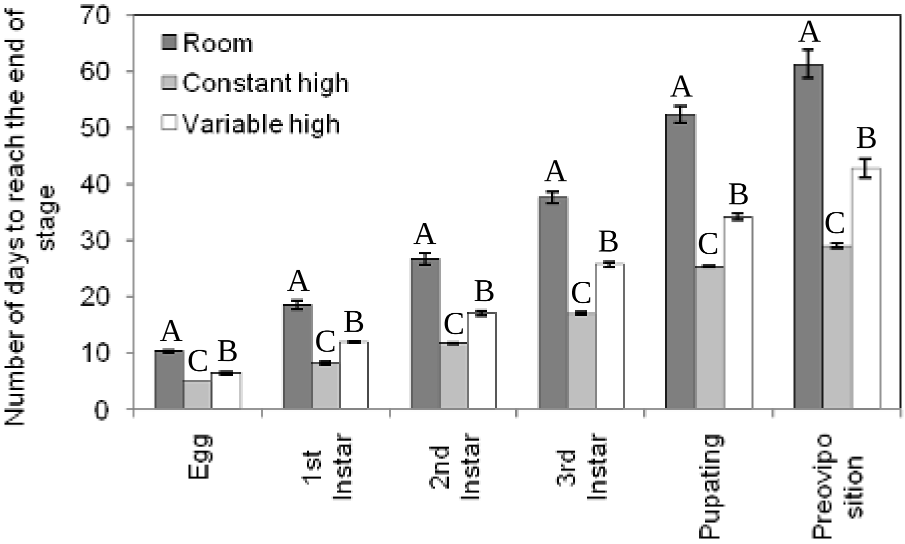


Figure V.8. Days to reach the end of each stage under different temperature conditions. Capital letters (A, B, C) in the figure represent results of Tukey's HSD test result; error bars are one SE.

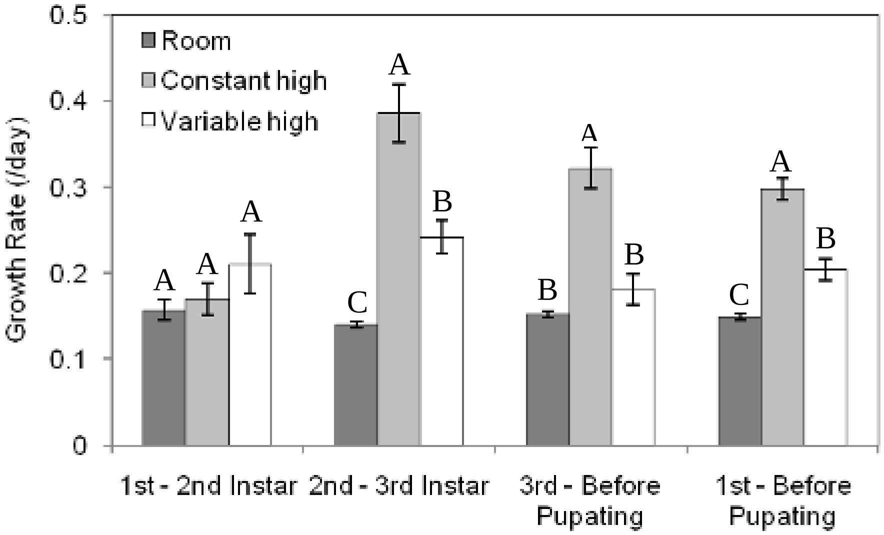


Figure V.9. Growth rate at each larval stage. Capital letters (A, B) in the figure represent results of Tukey's HSD test result; error bars are one SE.

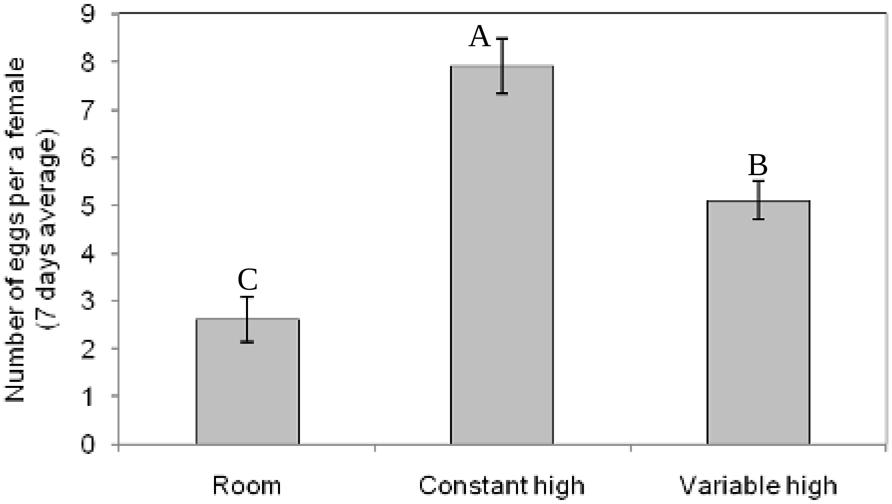


Figure V.10. Seven days average number of eggs per female per day. Capital letter, A, in the figure represent results of Tukey’s HSD test result; error bars are one SE.

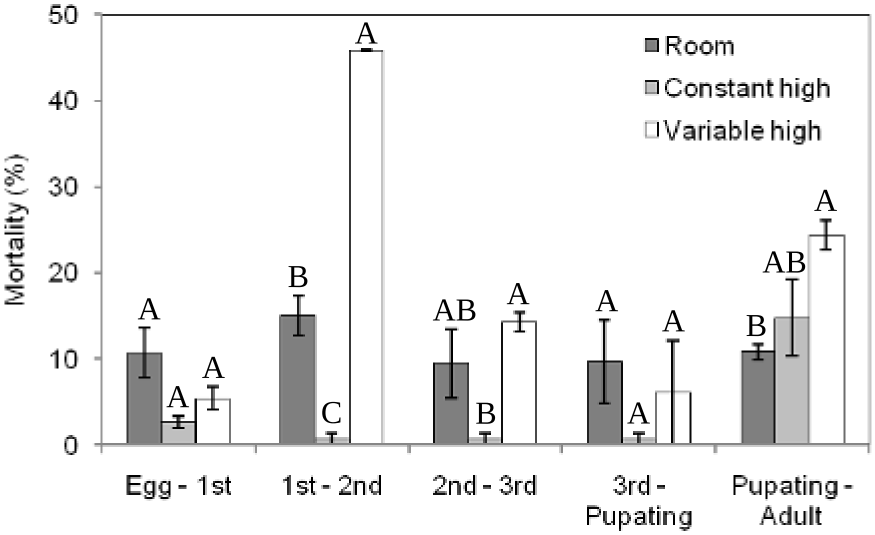


Figure V.11. Mortality at each stage. Capital letters (A, AB, B, C) in the figure represent results of Tukey’s HSD test result; error bars are one SE.

Section V-D. Rapid Evolution of Phenology and Range Expansion of an Introduced Insect

Background

The biological control program to manage tamarisk was initiated in the 1980's, and led to the selection of the tamarisk leaf beetle, *Diorhabda carinulata* (then identified as *D. elongata deserticola*) for initial field testing, beginning in 1998 (DeLoach et al. 2003). The first open field introductions of *D. carinulata* were made in 2001 at 8 test sites in Western North America. Beetles became established at more northern sites but failed to establish at sites south of the 38th parallel (Dudley et al. 2001, 2011). In subsequent years, some populations transported to lower latitudes, including the Virgin River well below this geographic threshold (Bateman et al. 2010), have established successfully, suggesting the intriguing hypothesis that rapid evolution of new life history traits has enabled this biocontrol agent to be effective in areas not previously anticipated to be suited for introduction.

Beetles undergo multiple generations per season in Asia and at some sites in North America (Dalin et al. 2010), then enter diapause as adults prior to host plant senescence and descend into the leaf litter beneath the tamarisk shrubs where they overwinter (Lewis et al. 2003). The source of beetles for most releases was near Fukang, China at 44°10' North latitude, and the introduced insects entered diapause early at southern locations; thus, it was likely that inappropriately timed diapause, induced by the shorter summer days experienced at lower latitudes, limited the number of generations per season and played a role in hindering establishment (Bean et al. 2007). Further work showed that adults enter diapause in the field 13 days after day lengths fall below 14 hr 39 minutes (Bean et al. 2007). This response to photoperiod allowed insects to remain reproductively active until mid August at a northern field site (LWY, Fig V.12) which most closely matched the Asian collection site in latitude as well as climate. At more southern sites diapause was induced by declining photoperiod as early as mid July. The phenology pattern for *D. carinulata* was in contrast to the general pattern of tamarisk phenology where the growing season is longer at more southern latitudes and green foliage is present later into the season (Dalin et al. 2010). The more southward beetles were introduced, the greater the asynchrony between life histories and host plant phenology.

Observations that beetles were reproductively active later in the season than had been noted in previous studies, and temporal expansion of *D. carinulata* activity was recorded at two widely separated field sites (PCO and LNV, Fig V.12). These observations led us to hypothesize that Critical Day Length for diapause induction (CDL, or the daylength at which 50% of the population has entered diapause) was evolving under the selection pressure brought about by phenological mismatch of *D. carinulata* populations with host plant availability and possibly other seasonal variables. The initial measurements of CDL made in the field as well as under controlled conditions in the laboratory, provided a starting point from which we could calculate evolutionary divergence from the ancestral state. We had good reason to believe that our initial measurements represented the initial state of CDL in *D. carinulata* after they were imported from central Asia. First, the field measurements were made early in the program, when insects had been in the field for less than 4 full seasons, while laboratory based trials were done with insects in culture derived from insects collected near Fukang, China and had never experienced open field conditions in North America, or had been in the field for less than 2 seasons (15). Second, initial field CDL measurements were nearly identical across a latitudinal gradient from 37°05' to 44°51' (37.08° to 44.85° N), indicating that measurable evolution had not yet occurred during the first 2-3 years in the field and those field based CDL measurements were consistent with the observed timing of diapause induction near the collection site in northwestern China (Li et al. 2000). Since we knew the initial state of the CDL we followed the trait in a population through time in an allochronic study.

In this study CDL was measured under laboratory conditions using cultures that were established from field collected insects at the end of the 2007 field season (when beetles began to enter diapause) or at the beginning of the 2008 field season after beetles were again reproductively active. Beetles were collected from five field sites ranging in latitude from 44°51’ to 33°00’ (Fig V.12). Beetles were cultured for at least one generation under laboratory conditions (19) prior to measuring CDL, in order to minimize phenotypic effects on diapause induction. CDL was measured under two temperature regimes; constant 25° C and a thermoperiod where temperatures fluctuated between 35° C (day temperature) and 15° C (night temperature). This temperature regime approximates summer temperatures in the arid interior of western North America and CDL results using this temperature regime most closely approximate CDL measured in the field.

Methods

Insect Sources Adult beetles were collected from field sites in Lovell, Wyoming (LWY) August 2007, Lovelock, Nevada (LNV) November 2007, Pueblo, Colorado (PCO) August 2007, Carp/Meadow Valley Wash, Clark County, Nevada (CNV) July 2008 and Artesia, New Mexico (ANM), July 2007 (Fig. V.12). Beetles collected from the LWY, LNV and PCO sites were the descendents of beetles released into the open field during the spring and summer of 2001. The CNV population resulted from colonization by beetles originally transported from the Sevier River to the Virgin River at St. George, Utah (Dudley and Bean 2011). Those from ANM were the descendents of beetles released there in 2005, which were collected from the LNV site. Beetles were held in culture as previously described (Bean et al. 2007) and used in experiments after 1-5 generations.

Determination of Field CDL Adult *D. carinulata* were collected weekly from the PCO site, frozen and later dissected to determine condition of the fat body and reproductive systems in both males and females. Individuals were scored as diapause, reproductive or undetermined as previously described (Bean et al. 2007). Developmental character state (reproductive vs. diapause) was the binary response, dependent variable which was plotted against ordinal date. The date when 50% of the population was in diapause was estimated using logistic regression and inverse prediction, which also yields 95% confidence intervals (JMP8, SAS 2008).

Field critical daylength was calculated by subtracting 13 days from the date of a 0.50 diapause probability and determining daylength on that calendar date using standard astronomical tables. The rationale for this formula has been described (Bean et al. 2007) but essentially it is that field CDL is first perceived, then measurable impacts on physiology, such as cessation of reproductive activity and increased fat storage, are manifested at a later time calculated to be 13 days.

Determination of Laboratory CDL CDL was determined for each population at constant 25°C and at a thermoperiod averaging 25°C and fluctuating with amplitude of 20°C (35°C/ 15°C). Chambers made the transition between the high (thermophase) and low (cryophase) temperatures over a 3 h period through a series of 2.0°C steps (35°C/ 15°C). Thermophase and cryophase were always of 9 hour duration, regardless of photoperiod. Photoperiod was superimposed onto the thermoperiod such that the initiation of photophase (lights on) exactly coincided with the initiation of warming in the transition between cryophase and thermophase. Temperature fluctuations of 20°C with a 25°C average are common throughout the interior of western North America in the early summer.

Results and Interpretation

At constant 25° C the LWY population retained a CDL identical to that initially measured while beetles from the other sites had shorter CDLs and notably the CNV collected beetles had a CDL 41 minutes shorter than the original (Table V.3). When a thermoperiod was used in conjunction with the photoperiod the changes in CDL were greater in magnitude (Table V.3). Again beetles collected at the LWY site retained a CDL closely matched with the original, but beetles collected from the southernmost site (ANM) showed a CDL that was 54 minutes shorter than the original (Table V.4) in contrast to the CDL measured at a constant temperature which was only 25 minutes shorter (Table V.3). CDL was reduced by 10-54 minutes depending on the population (Table V.4). Measurements made using field collected insects from the PCO site showed that the population entered diapause 15 days later than it had five years earlier (Fig V.13). This shift in diapause phenology can be attributed to a shift in field CDL of 23 minutes; from 14 hr 37 min (15) to 14 hr 14 min (this study). This shift corresponds closely to the shift measured in the PCO population under laboratory conditions. Under a thermoperiod the CDL of the PCO population was 14 hr 28 min, 25 min shorter than the initial CDL (Table V.4).

The initial releases of *D. carinulata* in North America included releases in eastern California (37° 05’ N, 118° 14’ W) where mistimed diapause induction was a factor preventing beetle establishment (Lewis et al. 2003, Bean et al. 2007). Field studies during the summers of 2001 and 2002 showed that beetles entered diapause early in the season (mid July) at a time when temperature and food resources (green tamarisk foliage), would have been ideal for population growth and this phenology asynchrony was considered important in inhibiting establishment. In contrast, beetles at the CNV site have a shorter CDL than originally measured (Tables V.3,V.4), are well established in the area, and remain reproductive in early August (data not shown). It seems likely that one trait, the CDL for diapause induction, is playing a fundamental role in the southern expansion of *D. carinulata* in North America. Species distribution models based on environmental factors are widely used but it is rare to pinpoint specific traits, such as CDL, which drive phenological responses to environmental factors (Chuine 2010).

This study describes a case of rapid evolution, which is evolution occurring within a few generations and having an immediate ecological impact, including allowing colonization throughout the Virgin River, well south of their original effective range, and presumably could facilitate colonization further south into the lower Colorado River. Evolution of CDL has been inferred in a number of introduced insect species, especially agricultural pests. In the case of the fall webworm, *Hyphantria cunea*, the CDL shifted by 14 minutes in 7 years in regions of Japan (Gomi et al. 2007). CDL has been shown to rapidly shift in response to climate change within the native range of the pitcher plant mosquito *Wyeomyia smithii* (Bradshaw and Holzapfel 2001). However, in all studies thus far the initial state of the CDL at the time of introduction was not known and the dynamics of range expansion and CDL evolution were not measured.

Future patterns of CDL evolution will be determined by the genetic composition of the introduced populations (Pujol and Pannell 2008, Futuyma 2010), natural selection pressures across the introduced range (Taylor and Spaulding 1988) and selective forces unique to expanding range margins (Thomas et al. 2001, Phillips et al. 2010). Of these we are particularly interested in evolution at the range margins. The tamarisk leaf beetle aggregates in response to a male produced pheromone (Cosse et al. 2005) and this pheromone is not produced by diapause individuals. Aggregation occurs after long or short flights and again, flight behavior decreases in diapause individuals (Bean et al. 2007). While insect phenology is known to be a function of CDL (Taylor and Spaulding 1988) in this case dispersal rate is also a function of CDL and at the southern range margin these two factors will work in concert to select for a shorter CDL. There has been great interest in the evolution of dispersal phenotypes but this is the first case, to our knowledge, where a shorter CDL for diapause induction can be treated as a dispersal phenotype.

A large scale dynamic process is now underway in southern Nevada and other regions in which the evolution of CDL will impact phenology, fitness and range expansion of an introduced insect species. CDL evolution across the range of *D. carinulata* in North America will provide an interesting case study, instructive for understanding the adaptation of organisms to new or changing environments. More critically for ecosystem management concerns, this example will be useful in the lower Colorado River Basin and affiliated drainages in the context of planning and implementing future biological control programs or in predicting the progress of the current tamarisk biological control program, where beetles are only slowly entering tamarisk infestations at lower latitudes (Bateman et al. 2010, Bean and Dudley 2011). This case study will also provide insight into the ecological challenges and evolutionary responses characterizing unintended species introductions and subsequent biological invasions. Adaptation to climate change will also require a shift in photoperiodic responses (Bradshaw and Holzapfel 2001) and this case is an example of that, even though southward movement of species is not an expected outcome of climate change.

Figures and Tables.

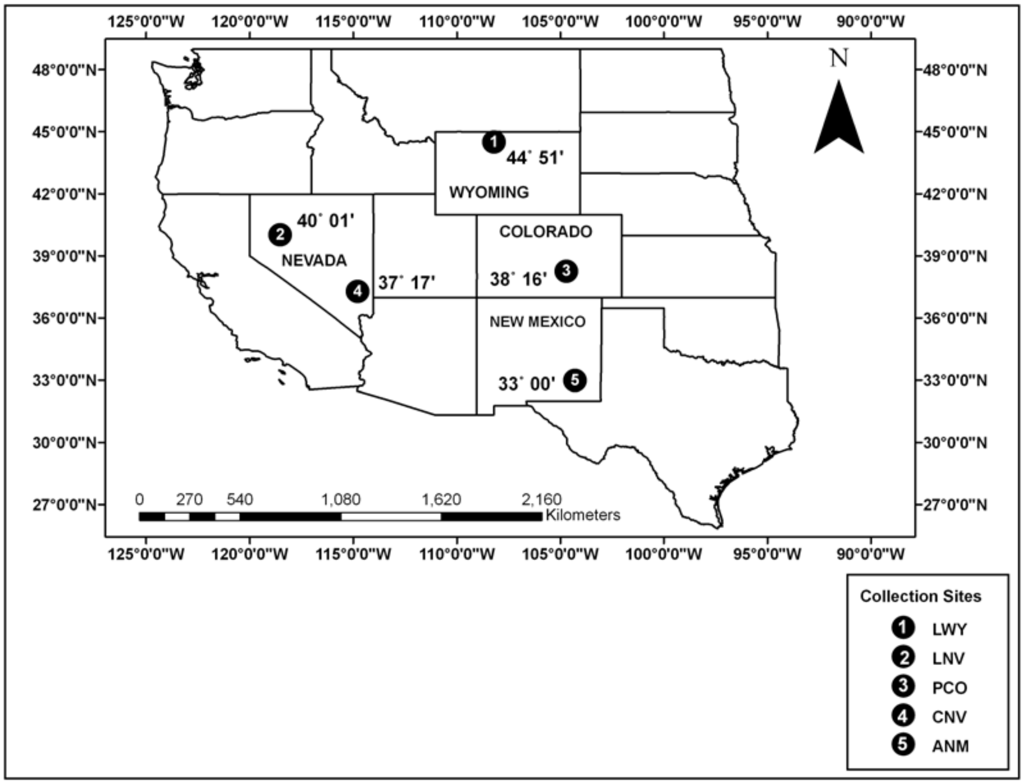


Figure V.12. Map of the western United States showing *D. carinulata* collection locations in Lovell, Wyoming (LWY), Lovelock, Nevada (LNV), Pueblo, Colorado (PCO), Meadow Valley Wash, Nevada (CNV) and Artesia, New Mexico (ANM) with latitudes shown next to each collection location.

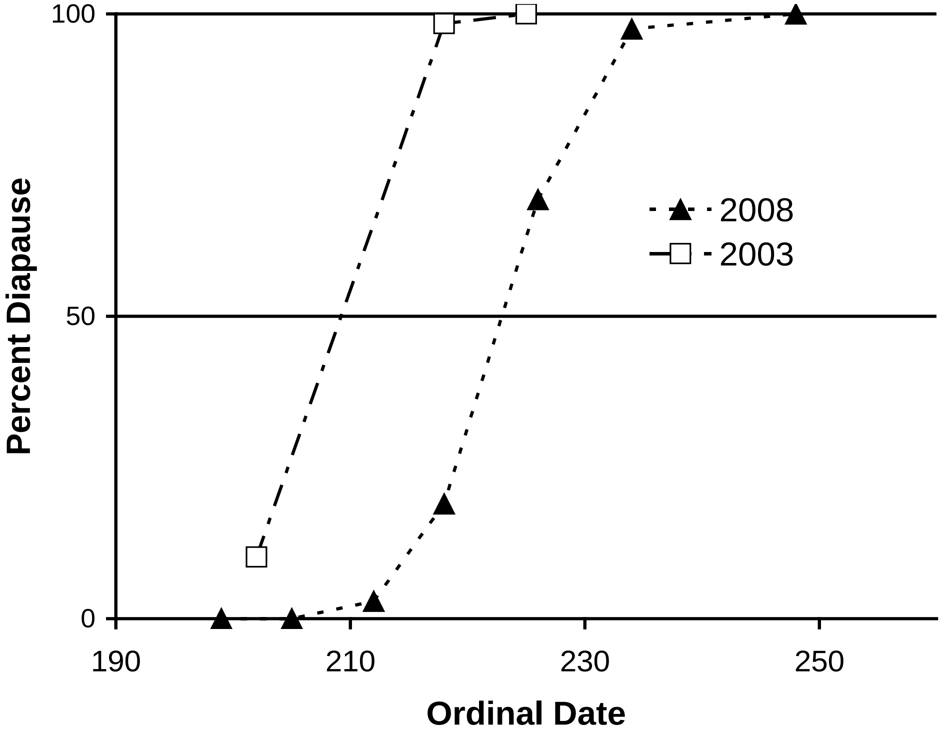


Figure V.13. Diapause incidence in the field at the PCO site. The 2003 values are from Bean et al 2007a. The 50% diapause is reached at ordinal date 223 (August 10, 2008) while in 2003 50% diapause was reached on day 207 (July 26).

Table V.3. CDL of Field Collected Populations

Temperature	Collection site	CDL (in hrs)	± 95% CI
25°C	[1]Original	15.13	15.23, 15.06
	LWY	15.13	15.21, 15.07
	LNV	14.97	15.01, 14.93
	PCO	14.86	14.93, 14.77
	CNV	14.45	14.51, 14.38
	ANM	14.72	14.80, 14.63
35°-15°C (average 25°C)	[2]Original	14.88	15.01, 14.78
	LWY	14.72	14.79, 14.64
	LNV	14.16	14.25, 14.08
	PCO	14.47	14.53, 14.42
	CNV	14.21	14.28, 14.13
	ANM	13.98	14.05, 13.92

[1] Original refers to the laboratory colony derived from field collected insects that came from three locations in western Nevada and eastern California as previously described (Bean et al 2007a).

[2] Original determination was made using a thermoperiod with an 18°C amplitude, 34°C-16°C, with a 25°C average (Bean et al 2007a)

Table V.4 CDL changes since the open field introduction of *D. carinulata* into North America

Strain	CDL (35°-15°C)	Difference (2008 minus original determination[1])
LWY	14.72	-0.16 (10 min shorter)
LNV	14.16	-0.72 (43 min shorter)
PCO	14.47	-0.41 (25 min shorter)
CNV	14.21	-0.67 (40 min shorter)
ANM	13.98	-0.90 (54 min shorter)
PCO (field)	14.23	-0.38 (23 min shorter)[2]

[1] The original determination of CDL was done under a thermoperiod with 18°C amplitude and an average temperature of 25°C while the CDL measured in 2008 was under a thermoperiod with amplitude 20°C. The CDL was originally measured at 14.88 hr (14 hr 53 min).

[2] The original determination of field CDL was made from data taken during the summer of 2003 at the Pueblo site, and was 14.62 hours (14 hr 37 min) (Bean et al 2007a).

Table V.5. Predicted change in the date of field CDL based on CDL values measured under laboratory conditions[1].

Field Site	CDL	Predicted date for 50% diapause in the field[2]		
		With original CDL	CDL from this study	Difference (Days shifted)
LWY	14.72	August 14	August 19	5
LNV	14.16	July 31	August 24	25
PCO	14.47	July 24	August 11	18
CNV	14.21	July 18	August 16	29
ANM	13.98	univoltine[3]	August 14	--
PCO (field)[4]	14.23	July 26	August 10	15

[1] CDL was originally measured at 14.88 hr under controlled conditions while field CDL was measured at 14.65 hr based on the average at 5 field sites (Bean et al 2007 b). Field CDL is calculated by subtracting 0.23 hr (14 min) from laboratory measured CDL.

[2] These are predicted dates given the CDL measured in the laboratory for each population. The original CDL predictions had already been made for field sites LWY, LNV and PCO and agreed closely with actual CDLs measured in the field (Bean et al 2007b) .

[3] Day lengths never reach 14.65 hours at the ANM site so using the original CDL there would be no reproduction in the first summer generation, making insects at this site univoltine.

[4] These data are taken from field measurements done in 2003 and again in 2008 and represent actual developmental status in the field. Field measurements closely match laboratory based predictions in 2003 (July 24 vas July 26, Bean et al 2007b) and again in 2008 (August 10 vs August 11, see Fig. V.13).

GENERAL DISCUSSION

Interpretation and discussion of the results of this research program are found in each of the Sections that comprise this full report, as these address specific objectives and research questions as independent sub-units. Here these results and conclusions are integrated into a more comprehensive consideration of how invasive plants affect, and can be managed in, riparian ecosystems in the Clark County region.

Non-Native Plant Invasions Invasive plants are part of the 'natural' landscape throughout Clark County and the surrounding Mojave Desert, and have substantial influences on biodiversity as well as ecosystem functions (Brooks 2006, Dudley 2006). In particular, invasion of riparian habitats by tamarisk species, of which there are at least 4 regionally (*Tamarix ramossisima, T. chinensis, T. parviflora, T. aphylla* and hybrids of these; Gaskin et al. 2002, Walker et al. 2006), has been long recognized as one of the most serious environmental impacts to these locally and globally endangered ecosystems (Sala et al. 1996, Busch and Smith 1995). The Virgin River is particularly affected by *Tamarix* spp. (hereafter referred to generically as 'tamarisk'), which achieves over 80% dominance in the system (Mortenson and Weisberg 2010), and in many segments is present as monocultures with close to 100% canopy cover by this plant. The effects of tamarisk invasion are widespread and well-known across western North America and have been the basis for many large-scale control programs requiring major investments by agencies and land managers (Shafroth et al. 2005, Taylor and McDaniel 1998). Some of these programs involve actions to restore native vegetation, but it is routinely observed that both control and restoration efforts lead to mixed, and often poor results (Bay and Sher 2008, Shafroth et al. 2008). In the Clark County region a major force driving invasive plant control, especially tamarisk control in floodplains and other wetland sites, is the reduction of flammable vegetation that can fuel the wildfires that increasingly occur in this region. An important, though secondary goal is to enhance habitat for wildlife species, while the Clark County MSHCP has expanded this mission to provide a policy framework focused on protection of federally endangered and other species of conservation concern (Covered Species). Both policies require replacement of tamarisk and other noxious invasive plants with alternative vegetation meeting these dual objectives, emphasizing the need to implement actions that lead to passive or active restoration of native riparian vegetation.

A persistent factor hindering successful recovery of natural communities is the re-occupation of weed treatment sites by noxious plants (D'Antonio and Myerson 2002). Such 'secondary invasions' can include re-growth and/or recruitment by the original target weed or by other species, some of which may have been suppressed by the target itself, but which can impede the recovery of native vegetation following weed control treatments. Many weedy species are at a competitive advantage when soils have been mechanically disturbed or native plants have been stressed by herbicides (Hobbs and Huenneke 1992, D'Antonio et al. 1995, Stylinski and Allen 1999). This is partly owing to their co-occurrence with other weedy plants like tamarisk in degraded ecosystems, such that removal of one opens space for the others to expand populations as a consequence of reduced competition. Substrate disruption also tends to result in nutrient releases from organic storage mechanisms, and many weedy species are adept (often relative to native taxa) at taking advantage of nutrient pulses to establish invasive populations (Vitousek 1990, Fenn et al. 2003).

Vegetative Response to Tamarisk Control Given the scale of many tamarisk treatments, particularly in floodplain systems such as the Virgin River, disturbances such as root-plowing or broad application of herbicides can be highly disruptive to typically unconsolidated riparian soils and to remaining native plants. In this project the clearing of tamarisk by standard mechanical and chemical methods strongly reduced the abundance, and by implication the negative impacts of this invasive shrub, and also tended to enhance the diversity of associated flora (53 vs. 32 species in untreated controls), including both native and non-native taxa. This was the case in both the upland sites where the NPS Exotic Plant Management Team has dedicated extraordinary effort to promoting recovery of these isolated but ecologically valuable spring wetlands, and in floodplain systems, particularly on the Virgin River where BLM has carried out extensive tamarisk removal programs, primarily for fuels reduction. However, these treatments also have led to increases by a variety of weedy plants, particularly forbs such as Russian thistle (*Salsola* spp.), burning bush or kochia (*Bassia scoparia*), bermudagrass (*Cynodon dactylon*). In the sandy substrates typical of the region, such weeds may maintain dominance for substantial periods and inhibit recruitment of native plants (Johnson and Fulbright 2008). The length of time since original treatment did correlate with decreasing abundance of these weeds, yet it is important to clarify that this is because of follow-up treatments by weed management professionals (see Section I). Incremental recovery of native communities would likely not have occurred without such intervention.

From a management perspective, it is important to rapidly develop protocols for treating such secondary invaders before they become serious problems in their own right, and in particular before they produce a large seed bank that subsequently creates long-term management problems. Interestingly, non-native grasses such as *Bromus* spp. and *Schismus barbatus* were somewhat more common in untreated tamarisk stands; these are not dominants, but the implication is that multiple invasive species may be favored by similar environmental conditions (referred to as an 'invasion complex'; Dudley 1994), while treatments may reduce these even while promoting increases by other invasive plants. Still, active intervention will routinely be necessary to manage weedy that might interfere with conservation goals. To this end, we tested a variety of herbicide treatments against another noxious weed, Russian knapweed (*Acroptilon repens*), that is a recent invader at Ash Meadows National Wildlife Refuge, with some incipient populations becoming established within Clark County (as described in Section IV, this report). Winter dormancy treatments indicated that aminopyralid, a relatively new herbicide with low non-target toxicity may provide effective control for this, and presumably other secondary weeds since it is a broad spectrum herbicide. In a later section we discuss tamarisk management approaches that may reduce the incidence of this and other disturbance-dependent weeds in promoting ecosystem recovery.

Concerns regarding responses by both native and non-native plants to tamarisk treatments further highlight the value of active restoration to potentially preclude secondary invasions and facilitate recovery of intended target vegetation. In our study it was difficult to assess the effectiveness of restoration actions, as these are only undertaken in a minority of weed control projects and thus there was inadequate statistical power to assess relative effectiveness of restoration tactics. Likewise, our research team initiated restoration treatments at five Virgin River sites, testing a variety of tactics including planting of *Salix* spp. *or Populus fremontii* 'poles' vs. rooted plants, deep vs. shallow planting, deep planting of *Salix* 'wattles' (multiple cut stems bundled together and installed horizontally fully below the surface), seedlings vs. year-old *Acacia* and *Prosopis* plantings, inoculation with prepared mycorhizae, nitrogen fertilization, soil moisture amendment with colloids or clay compounds, and also livestock exclusion experiments. These, along with many BLM restoration efforts with mesic (*Populus, Salix*) and xeric species (*Prosopis, Atriplex*) were largely destroyed by flooding in 2010 before they had achieved sufficient maturity to evaluate effectiveness of restoration approaches, and so were inconclusive. These unfortunate results highlight the need to fully consider how hydrological processes may affect restoration success, a factor commonly associated with failure of riparian restoration programs to achieve original objectives (Palmer et al. 2009). Achieving these goals is generally less successful than anticipated, particularly because long-term, self-sustaining biotic assemblages depend on re-creating complex, inter-linking ecosystems that are both resistant to natural and anthropogenic disturbances, and resilient so that natural recovery processes such as primary and secondary succession can proceed with little intervention (Poff et al. 1997, Zedler and Calloway 1999).

Biodiversity Response to Tamarisk Control Thus, traditional methods of tamarisk removal have mixed effects, effective in meeting some objectives, increasing diversity and abundance of native plants and reducing the likelihood of wildfire (sparser distribution of plant biomass, or 'fuels' in treated area). However, a fundamental conclusion is that, in general, conventional treatments have the undesirable effect of increasing the abundance of other noxious, non-native species that can interfere with native vegetation recovery. Furthermore, the effect of these treatments on wildlife associations, at least in the floodplain ecosystems where surveys were conducted, is more clearly negative (while recognizing that the goal was primarily fuels reduction, and only secondarily for habitat enhancement). Overall avian abundances were three times greater in untreated plots (dominated by tamarisk) than in plots where tamarisk had been removed, and on a species basis, the same relationship held across the large majority of bird species (see Section II). These included insectivores, seed and fruit feeders, and other foliage and ground gleaners, birds that tend to be positively associated with vegetation density (Skagen et al. 1998, Anderson et al. 2004). Birds tending to be more commonly found in treated plots were those associated with more open habitats for pursuing prey or foraging for seeds or insects on plants found in open areas (e.g. shrikes, kingbirds, phainopepla, mockingbirds, Brewer's and lark sparrows, Say's phoebe, etc.), as there was greater open ground present in these areas.

These surveys were only 2 to 4 years after treatments which were nearly all initiated following the destructive 2005 floods, so improvement could conceivably occur over time. But the relatively poor recovery of large-statured, woody native plants suggests that conditions for wildlife will not dramatically improve without active restoration of more desired vegetation.

While tamarisk is well known to be generally inferior to native riparian vegetation for supporting wildlife, it does provide adequate habitat for many species (Bateman et al. 2010, Ellis 1995, Anderson & Ohmart 1977, Fleishman et al. 2003, Rice et al. 1984, Hunter et al. 1988). The presence of at least some native vegetation consistently supported significantly greater abundance and diversity of avian species (Appendix 1, this report), as well as bats (Appendix 2, this report), herptiles (Bateman et al. 2010) and small mammals (Ostoja and Bateman, unpub. data) at our Virgin River study areas. This is likely to be valid for MSHCP Covered Species such as southwestern willow flycatcher (SWFL), although densities were insufficient to evaluate such relationships statistically. Anecdotally, willow flycatchers (which were clearly SWFL, but Clark County protocol requires multiple sightings to validate sub-species identification) were documented only in untreated plots; two of the four individuals were, however, in plots with native willow mixed amongst the dominant tamarisk. Important information can be acquired by comparing other birds that have similar habitat relationships, and the yellow warbler (*Dendroica petechia*) is considered a good proxy for SWFL (see Appendix 1). Its numbers were approximately a third higher in mixed vegetation stands than in tamarisk monocultures, even though the Mixed vegetation types was still heavily dominated by *Tamarix* spp. It is worth noting that brown-headed cowbirds, a nest parasite of conservation concern because of its potential impacts to special status species, were far more abundant in the untreated plots than where tamarisk was removed, highlighting the general beneficial value of reducing tamarisk abundance if native vegetation can be retained or increased. At the same time, large-scale removal of tamarisk is likely to have some deleterious effects on bird communities, at least in the short term, so it remains important to consider timing and approaches to control efforts that will minimize direct negative effects.

All wildlife studies conducted as direct or indirect elements of this MSHCP project point toward the critical value of enhancing the relative abundance of native woody plants that provide greater architectural heterogeneity, more food resources and lower fire risk. Thus, habitat restoration focused on providing, or increasing abundances of native trees and shrubs (*Salix* spp. and *Populus fremontii* in mesic, lower floodplain terraces; *Prosopis* spp. and *Acacia greggii* on higher terraces) to increase plant diversity is a desirable element of river management strategies, despite the lack of capacity to evaluate such approaches within the constraints of this project.

Biological Control of Tamarisk The introduction of the tamarisk leaf beetle (*Diorhabda carinulata*) into the Colorado River basin in 2001 provided the basis to start planning for eventual introduction and/or dispersal into Clark County riparian and wetland ecosystems, particularly the Virgin River and the mainstem Colorado River. Initially in this project we simulated the effect of this specialist herbivore using low-dose herbicide applications to experimental plots to evaluate responses of vegetation composition and structure, fuels and fire risk, and wildlife habitat associations, once biological control with *Diorhabda* (biocontrol) was implemented in this ecosystem. During the course of this project, however, *D. carinulata* was first introduced by resource managers from our biocontrol research site in the Sevier River (Delta, Utah) into the Virgin River watershed in St. George, Utah. The beetles established locally during the 2006-2007 field seasons, then in 2008 proceeded to disperse unassisted in multiple directions, including along the Virgin River in Arizona and Nevada, as well as southward to the Colorado River where it is now present within the Grand Canyon (see Section V).

The presence of biological control agents in southern Nevada changes the nature of all future tamarisk management, as biocontrol will inextricably be a factor in weed management and system responses for the foreseeable future. This unanticipated insect release provided the context and need for monitoring its impact and effectiveness as a weed management 'tool' for invasive plant suppression and wildlife habitat restoration. Currently (Spring 2011) the tamarisk leaf beetles are present throughout the Virgin River watershed from Zion National Park to within 5 km of Lake Mead, as well as throughout Meadow Valley Wash/Creek, in much of Beaver Dam Wash, and in the Muddy River from its confluence with Meadow Valley to the north edge of the Overton Wildlife Management Area. No beetles are yet present at the Warm Springs NWR, but colonization is anticipated during 2011, as is colonization of Lake Mead tamarisk infestations from both the Virgin and Muddy Rivers, as well as from the east via the Colorado River.

The biocontrol process, life cycles and initial impacts to target plants are outlined in Section V but in summary, *D. carinulata* exhibits two and possibly a partial third cohort (generation) in this region prior to induction of diapause and adult insect entry into the litter for over-wintering in response to declining daylength (Bean et al. 2007). High densities achieved by larvae and adults lead to near-complete defoliation of plants within 2 to 3 weeks. Re-growth is fairly rapid, new foliage being produced within 4 to 6 weeks, while repeat defoliation over the course of 3 or more years can lead to target plant mortality as seen in other regions (Dudley and DeLoach 2007, Dudley and Bean 2011, Hultine et al. 2010). Mortality occurs when herbivore stress limits the host plant's capacity to acquire and store nutrition, as indicated by soluble carbohydrate levels in stem or crown tissues

(Hudgeons et al. 2007; Sections III and V, this report). This mechanism is particularly effective late in the growth season when plants no longer have the capacity for re-foliation to compensate for metabolic reserves exhausted by herbivore-induced foliage loss; we have documented a small number of target plants that have already died via this mechanism.

BioControl and Evolution of New Relationships Interestingly, our studies have shown (Section V-D) that *D. carinulata* has undergone natural selection in the southern Nevada region to better synchronize developmental changes (over-winter diapause) with this southern latitude and host-plant growing conditions (Dalin et al. 2010, Bean et al., submitted). Evolution of the seasonal organization of life history (phenology) provides an important step in the movement of organisms into new ranges (Thomas et al. 2001), including those intentionally introduced for biological control (Hufbauer and Roderick 2005), in biological invasions (García-Ramos and Rodríguez 2002), or species adapting to climate change (Chuine 2010, Gomi et al. 2007, Bradshaw and Holzapfel 2001). The primary environmental cue used by *Diorhabda* spp. to synchronize life history events with seasonally available resources is photoperiod, a precise indicator of the calendar date and thus the onset and decline of resource suitability (Bean et al. 2007). Evolution of the day length requirement for continuous development, termed critical day length (CDL), has been documented in a few insect species and inferred in others (Riedl and Croft 1978, Pujol and Pannell 2008). There are no prior cases where initial CDL is known and where evolution is tracked as an introduced species moves into a new range.

In this project we found that after five years, or about 10 generations, CDL (technically the day length at which 50% of a population has entered diapause, or over-winter dormancy) has decreased by 10 to 54 minutes in *D. carinulata* populations collected from four field sites across a latitudinal gradient. The furthest south population, which showed the greatest evolutionary adaptation to daylength, was from Meadow Valley Wash, Clark County, NV, and derived from the population introduced into the Virgin River watershed in 2006. These changes allow reproduction later into the season at more southern latitudes, enabling beetles to establish in regions where they were previously restricted by inappropriately synchronized diapause phenology. This is an example of phenology evolution enabling a range shift, demonstrating how rapid evolution of a physiological trait is an essential component for movement into new ranges (Thomas et al. 2001, Phillips et al. 2010). This evolutionary process, which was anticipated but not at the rate that we observed, facilitates *D. carinulata* establishment in southern Nevada, and we anticipate that continued colonization into the lower Colorado River region will occur in the next few years as selection enables populations survival at these latitudes, and thus preparations for defoliation via biological control in the region should be taken into account in all future tamarisk management and riparian restoration programs.

Baseline Monitoring and Application to Restoration A major focus of this research project was directed toward documenting the process of biocontrol establishment and the responses of wildlife and ecosystem functions to the changes that herbivory causes, or will cause, to natural resources in regional riparian systems. These are addressed in Section V and Appendices I through III as part of Objective 5 of this Monitoring Research project, but the timing of the research relative to the MSHCP project timeline means that the majority of results are not ‘experimental’ (that is, documenting responses to this natural experiment involving a novel herbivore), but instead will comprise an extensive baseline dataset for documenting and understanding these changes as they occur over the course of the next several years (Bateman et al. 2010, Dudley and Bean 2011). Monitoring of tamarisk biocontrol is underway in several areas of the western U.S. (DeLoach et al.

2005, Dudley et al., in press), but these are very limited in nature (Hultine et al. 2010) and the studies underway in the Virgin River watershed will be the only comprehensive evaluation of biodiversity and ecosystem responses to tamarisk biocontrol to-date.

The current program also has a larger context, as providing the only means of resolving the legal dispute (Center for Biological Diversity and Maricopa Co. Audubon Society vs. USDA APHIS and the USDI Fish & Wildlife Service) concerning non-target impacts of biological control of tamarisk that has caused federal regulators to stop tamarisk programs nationwide, as well as other national programs intended to develop and apply biological control for environmental weeds (Dudley and Bean 2011). The conflict over tamarisk biological control and risks to SWFL is centered on the Virgin River because of the speculated risk that defoliation by *Diorhabda* spp. may pose risk if it occurs during nesting of SWFL (Paxton et al. 2011, Bateman et al. 2010), and this is the basis for the legal proceedings that remain on-hold until resolution is achieved through negotiations among the litigants (Dudley and Bean 2011). Resolution can only come through presentation of data showing the responses of SWFL and other wildlife species, vegetation and ecosystem functions to introduction of biocontrol agents, including the feasibility of restoring native plant species successfully.

The multi-agency, multi-disciplinary monitoring program that we have put into place, including surveys of birds, small mammals and bats, reptiles and amphibians, arthropods, water resources and other ecosystem elements (Bateman et al. 2010) is intended to provide the scientific framework for such a resolution. These data will also provide the information base for a larger program facilitated by the Tamarisk Coalition (Dudley is a former Board member of this NGO) and to be supported by the Walton Family Foundation and other leveraging partners, to implement riparian habitat restoration in the Colorado River Basin. The purpose of restoration is to enhance native habitat suitable for supporting the SWFL along with other federally listed and/or regionally sensitive wildlife species. The restoration program is initially focused on the Virgin River watershed as the only place where *Diorhabda* spp. beetles are found within the breeding range and designated Critical Habitat of the endangered flycatcher sub-species. Planning is underway to apply the information we are generating towards design and implementation of riparian restoration for the Virgin River floodplain, and for tributary systems located within Clark County.

Tamarisk and Fire Before discussing riparian restoration in the region, it is necessary to also address the role that fire has in the relationship between tamarisk invasion and riparian community condition. Wildfire in Clark County and in the Virgin River watershed is one of the key stressors of ecosystem integrity and sustainability, as noted by the recent watershed study conducted under the guidance of the Army Corps of Engineers (Estergard 2008). It is well known that heavy tamarisk cover presents a serious fire hazard, yet the detailed relationships among fire (both wild and prescribed), tamarisk invasion, biological control and riparian ecosystems remain little documented (Brooks et al. 2008).

The build-up of hazardous fuels by tamarisk and associated weedy plants has become a significant fuel management problem as less flammable native species are displaced (Brock 1994, Busch 1995). Land managers remove tamarisk to reduce fire risk because intense fires are driven by the high stem density and deep litter found in dense stands (Harms and Hiebert 2006, Swanson and Gilgert 2009, Conlin 2010). Fire behavior in tamarisk stands is generally considered more extreme than in native riparian vegetation because native species are considered non-flammable and fires have become more common with tamarisk invasion (Gregory et al. 2003, Bêche et al. 2005). In other shrublands (e.g. chaparral) vegetation can produce flame lengths of 30 m under conditions of high wind and slope (Conard and Regelbrugge 1993, Riggan et al. 1994), but in our Valley of Fire experimental burn (Section III-B) flame lengths >35 m were documented in healthy as well as desiccated tamarisk stands (by herbicide to simulate *Diorhabda* defoliation), even under non-windy conditions. Flame lengths up to 43 m have been reported elsewhere (Racher et al. 2001), and that was in tamarisk stands that were vigorous and green. Many tamarisk-fueled wildfires occur in winter or when moisture content in the deciduous vegetation is dry, but the fact that tamarisk burns intensely even during the growing season is a primary factor in hypothesizing that tamarisk changes riparian systems from high-moisture barriers to fire spread into pathways, or 'wicks' for fire spread (Dudley 2008, Lambert et al. 2011). Environmental factors play a large role in this relationship, as fire intensity was strongly enhanced by drier, hotter and windier conditions similar to previous burns in tamarisk stands conducted during summer months (Howard et al. 1983).

There are, however, no empirical data on the fire behavior of native riparian vegetation for comparison to tamarisk nor to explain the mechanisms underlying the increase in riparian fires observed with tamarisk invasion. The foliar flammability experiments confirm the greater ignitability of tamarisk foliage relative to native material, as it ignites (chars or flames) more quickly than does native foliage. Although live tamarisk foliage contains only 10-15% lower tissue moisture than cottonwood (*Populus fremontii*) or willow (*Salix* spp.), all of the tamarisk samples produced flame while only half of the cottonwood samples, and none of the willow samples produced flame in a muffle furnace at 650 ˚C. When desiccated, tamarisk only produced flame a third of the time, while cottonwood and willow produced flame two thirds of the time, supporting high flammability of native riparian areas during periods of extreme drought. These results support the observations that the increase in riparian fire in tamarisk dominated areas is driven by the greater flammability of live foliage relative to native species (Weisenborn 1996, Racher et al. 2001, Lair 2006).

To gain a broader representation of the role of fire in tamarisk-occupied ecosystems, a survey of recent riparian burns in the southwestern U.S. (Section III-A) indicated a positive feedback dynamic between tamarisk density and fire incidence (cf. Brooks et al. 2004). Native vegetation consumption by wildfire and subsequent mortality increased in direct relation to tamarisk density across survey sites. Not only is tamarisk more flammable than native species, but also native plants are much less tolerant of fire [tamarisk readily grows back from root crowns following even severe fire (Section III-A,B)], and the greater the tamarisk relative density, the higher the native plant mortality that occurs in a density dependent manner. Many riparian species can re-sprout following fire, as the vegetative propagation capacity that is an adaptation to regular flooding disturbance (Naiman and and Décamps 1997, Ellis 2001) also enables re-growth from moderate fire damage. Re-sprouting is a general response to severe disturbance by many woody plants (Bond and Van Wilgen 1996). However, the ability of riparian species to resprout decreases with fire intensity (Ellis 2001; Section III), and species such as Fremont cottonwood are relatively sensitive to fire (Rood et al. 2007).

Thus, tamarisk promotes its own post-fire recovery and expansion, thus further displacing native vegetation, illustrating the management importance of reducing the relative abundance of tamarisk in regional ecosystems. As tamarisk dominance increases, the risk of wildfire increases to the detriment of associated wildlife, and it increasingly seems that wildfire in previously fire-resistant desert riparian areas poses one of the most serious risk to wildlife that that depend on these ecosystems (Dudley et al. 2000), including MSHCP Covered Species. This was pointedly made clear by the July 2010 wildfire at the Warm Springs NWR in which a fire, partially fueled by tamarisk, destroyed active nests of southwestern willow flycatchers (R. Johnson & D. Szydek, Southern Nevada Water Authority, pers. comm.). There is now concern that the endangered bird will abandon nesting at this wildlife reserve, although one individual was observed at the site in 2011 (R. Johnson, pers. comm.).

Tamarisk Biocontrol and Fire The feeding mode of *Diorhabda* spp. beetles is to scrape the epidermis of green leaf and stem tissues, thereby causing water loss from, and desiccation of foliage over a fairly short period (Lewis et al. 2003, Snyder et al. 2010)]. When large areas of tamarisk are so defoliated, massive browning of vegetation it raises serious questions concerning the potential for elevated fire risk. This is likewise a concern in Clark County, and particularly on the Virgin River where tamarisk forms near-monocultures in much of the floodplain, and where wildfire is a common threat. Along the Colorado River in central Utah this cause public safety concerns and BLM resources managers actively removed many trees because of the speculative threat. Our lab studies suggested that this may not be the case, but evidence from fires under field conditions are necessary in order to objectively answer this question. Low fuel moisture generally enhances fire intensity, rate of spread and fire risk because dry fuels are more easily ignited (van Wagtendonk 2006).

Therefore, in addition to the laboratory studies, we examined the influence of herbivory desiccation on fire behavior both where *D. carinulata* was already established and where herbivore defoliation was simulated with herbicide. While the fire intensity index (a measure that integrates temperature and duration) indicated that desiccated plants do burn significantly more intensely, the difference between treatment plants (by both insect feeding and herbicide treatment) was much smaller that we had anticipated (Section III-B). This again provides evidence that, while green and 'browned' plants still pose an important fire hazard, the impact of biocontrol does not substantially increase that hazard level. The surprising result may be related to volatile organic compounds in live plant tissues, which in other fire-prone vegetation are known to increase burn behavior (Keeley et al. 2005). Faster rates of fire spread were observed in the simulated herbivory treatment where the majority of fine fuels were intact, than where *D. carinulata* grazing had reduced live canopy cover over several seasons; live canopy cover was reduced by 90% over three seasons at a site adjacent to the Humboldt site (Pattison et al. 2010). Higher tamarisk density provided greater fuel continuity, which increased fire intensity and flame lengths. Similar to chaparral systems, the combination of high fire intensity and flame lengths promoted greater fuel consumption or vegetation removal (Menges and Deyrup 2001, Keeley et al. 2005, Hood et al. 2007).

Over time the threat of wildfire will decline. Within a single growing season, several beetle defoliation events can occur, followed by production of new foliage after several weeks (Dudley 2005). With each defoliation event, however, less foliage is produced; 40% less canopy cover was observed following an initial defoliation events, and cover continued to decline with continued herbivore over a 3+ year period (Pattison et al. 2011). Furthermore, herbivory reduces the production of fine fuels and enhances litter decomposition, fire intensity may decrease over time (Uselman et al. 2009) similar to what is observed in bark beetle-affected conifer forests (Knight 1987, Bebi et al. 2003). The initial tamarisk defoliation event, or first year of *Diorhabda* spp. colonization, is therefore likely to be the only time when biocontrol will increase fire risk, while flammability decreases over time as *Tamarix* spp. lose the physiological capacity to replace damaged leaves.

These dynamics further suggest that biocontrol may in the long term lead to improved conditions for native vegetation, vis-à-vis its influence on fire intensity and destructive capacity. As tamarisk biomass and vegetation cover incrementally decline over multiple herbivory bouts, not only is space opened for enhancing growth of remaining native plants, but if fire does occur, the lower intensity of such burns will mean that the natives may not experience such heavy mortality. Already in some areas such a much of the lower Colorado River, land managers do not expend effort of revegetating with native trees because of the probability that tamarisk-fueled fire will destroy their efforts, but biological control may shift this dynamic over time to make restoration feasible, even with residual tamarisk still present. In other words, we potentially have a synergistic relationship with fire and herbivory will shift the system from one dominated by tamarisk to one where the coexistence of natives is possible, as increased tamarisk mortality over time will decrease biomass and fire intensity, allowing native survival and recovery. Eradication of tamarisk may not be possible given the irreversible nature of the hydrologic changes that promoted its invasion, but future coexistence of native and non-native vegetation may allow recovery of some habitat value to tamarisk occupied ecosystems.

Fire, Biocontrol and Tamarisk Mortality Prescribed fire is often used to reduce tamarisk biomass, but plants readily resprout from its root-crown and nutrients released by fire stimulate its regrowth (Ohmart et al. 1988, Busch and Smith 1993, Holht et al. 2002) , so fire is generally a poor tool for actually killing this target. The introduction of an important herbivore, however, may change this dynamic (Brooks et al. 2008). The interplay between fire and herbivory can enhance mortality in other systems, such as in various conifer forests subjected to bark beetle infestations (Lichtenthaler 1998, McCullough et al. 1998, Menges and Deyrup 2001). This suggests that a coupled management strategy of *D. carinulata* herbivory and prescribed fire may be more effective than either strategy alone.

In our studies, *Tamarix* mortality was influenced by a combination of fire intensity and physiological stress (Section III-C). In northern Nevada where *D. carinulata* has been a part of the system for several years, augmentation if litter contributed to higher fire intensity and mortality, corroborating studies that show litter and understory weeds associated with *Tamarix* spp. produce high surface fire intensities and greater tissue damage (Bradstock and Auld 1995). In addition, we found summer weather conditions (high ambient temperature and low relative humidity) promoted higher fire intensity and greater overall mortality in tamarisk, similar to that observed by others (Bevins et al. (1980, Howard et al. 1983). Herbivory desiccation of tamarisk foliage marginally increased fire intensity, but the relationship between fire intensity and mortality was much weaker than the relationship between starch content and mortality.

Diorhabda carinulata herbivory causes physiological stress to its host plant, making it more susceptible to additional treatments, such as fire. Herbivory reduces carbohydrate stores in tamarisk (Hudgeons et al. 2007), so energy stores are no longer available to replace leaf material consumed by the beetles, or to maintain growth and vigor. Higher post-fire mortality was observed in plants with lower root crown starch content (Section III-C). Plant phenology also influenced recovery; carbohydrate reserves fluctuate over the growing season with the lowest levels reached during the height of the growing season (June-August) when all energy is devoted to growth (Bartley and Otto 1961, Cords and Badiel 1964). Therefore, the higher mortality observed in the August burn treatment at Humboldt is also related to lower seasonal energy reserves. While the Valley of Fire experimental burns cause only trivial target mortality, mortality was not the intention of that experiment, which simply was testing the effects of herbicide-simulated herbivory on fire behavior. Nonetheless, prescribed fire could be used as simply one of three management treatments to control this invasive weed, as biocontrol, herbicide application and fire could all be part of a strategy for enhancing mortality and reducing its negative ecological impacts in a cost-effective manner, particularly when combined with a strategic approach to also restoring native vegetation in riparian ecosystems.

RESTORATION FOR ENDANGERED SPECIES HABITAT

As discussed in each project section above, the over-arching goal of this and other projects in the region is to facilitate the rehabilitation of native riparian habitat for the benefit of Covered and other special status wildlife species. It proved infeasible to evaluate native plant restoration trials and implementation projects regionally owing to flood disruption of sites before adequate maturation had been achieved, however the guidelines for conducting riparian restoration are already reasonably well-known, including restoration following tamarisk control (Palmer et al. 2009, Shafroth et al. 2008, Lair 2006). In southern Nevada and surrounding landscapes, it remains important to determine how to apply these prescriptions to the circumstances present, particularly with respect to both the specific objectives of restoration [the primary one being provisioning habitat for southwestern willow flycatcher (SWFL) and other special status species] and the hydrological status of the watershed and floodplains. These issues have been outlined in a recent article (currently *in press*) that specifically addresses the need for, and effective approaches to riparian restoration in the Virgin River watershed. That portion addressing restoration is excerpted here, and may serve as a partial framework for Clark County riparian restoration. From Dudley and Bean (2011):

Restoration Goals Restoration Ecology is a subset of Conservation Biology, a multi-disciplinary field that seeks to promote biodiversity protection along with rehabilitation of ecosystem functions that sustain both environmental and economic values in human-altered ecosystems. Restoration of riparian ecosystems has been practiced for years with varying success (Palmer et al. 2009), primarily for natural resource enhancement and to promote so-called ecosystem services such as bank stabilization and erosion reduction, water conservation, effluent sequestration, and aquatic and terrestrial wildlife production (Ehrenfeld 2000). Less often, the primary objective is to enhance populations of protected species, but this is an important secondary objective in promoting restoration programs. Achieving these goals is generally less successful than anticipated, particularly because long-term, self-sustaining biotic assemblages depend on re-creating complex, inter-linking ecosystems that are both resistant to natural and anthropogenic disturbances, and resilient so that natural recovery processes such as primary and secondary succession can proceed with little intervention (Poff et al. 1997, Zedler and Calloway 1999).

Restoration to specifically create habitat for a single endangered species, particularly in the context of a weed management program, is a narrow and atypical goal for ecosystem rehabilitation efforts (Goodwin et al. 1997). In riparian systems, wildlife species may have very specific requirements for habitat selection that are hard to re-create, and these tend to be functional ecosystem parameters (e.g. vegetation density, patch size, moisture conditions) rather than simply the presence of a target plant species growing at a site; you may build it…but they won’t necessarily come. Avian nest site selection is a particularly complex decision-making process using indirect environmental cues to indicate that future conditions will lead to breeding success (sufficient food resources, low incidence of predators, etc.), and often prior knowledge (site fidelity) that the site has been successful before (Fretwell and Lucas 1970, Stamps and Swaisgood 2007). The general habitat associations of the willow flycatcher are known (dense, multi-level vegetation adjacent to open water or saturated soils), but the precise cues it uses for nest site choice are poorly understood (Sogge 2000). Furthermore, the type of vegetation it prefers is transitory, with habitat suitability declining as vegetative succession matures to larger trees and a less dense understory (Sogge et al. 2003). Thus, riparian restoration should be done on a landscape scale, mimicking the natural successional processes and promoting conditions in which there is a shifting mosaic of vegetation patches with areas of early successional stands consistently present (cf. Cardinal and Paxton 2005). It is generally insufficient to just get some green plants growing.

In the case of the southwestern willow flycatcher in tamarisk-infested riparian areas, the basic goal is to ensure sufficient habitat is available which meets nesting requirements so that biocontrol-induced decline in tamarisk suitability can be mitigated by presence of nearby vegetation as a refuge or alternative habitat element. The question is how to provide those requirements. Assuming that key environmental features (stand extent, adjacent standing water or saturated substrate, arthropod availability, etc.) are suitable, it should be relatively easy to establish fast-growing natives such as willows to supplement the suppressed tamarisk. However, willow flycatcher specialists acknowledge that such efforts to-date have not been successful in facilitating occupation (E. Paxton, pers. comm..). Large-scale native tree horticulture near the lower Colorado River by the U.S. Bureau of Reclamation has attracted some sensitive native birds as the vegetation matures, particularly yellow-billed cuckoo (T. Olson, unpub data) but willow flycatchers have not been similarly attracted to this novel feature. Anderson and Ohmart (1982) caution, however, that many years are required before desert riparian restoration success or failure can be reasonably judged.

On the other hand, there are at least two situations where unassisted establishment of native plants (albeit with *Tamarix* spp. present in the mixed composition stands) has promoted important increases in willow flycatcher occupation. At the Salt River and Tonto Creek inflows to Roosevelt Lake in Arizona, inundation followed by receding lake level during a drier period in the 1990's allowed establishment of willows, cottonwoods and some tamarisk, which led to a four-fold increase in the number of SWFL territories in the area (Newell et al. 2003). Likewise, at Elephant Butte Reservoir on the Rio Grande in New Mexico, when a large stand of tamarisk was inundated and subsequently replaced by native willows as levels stabilized a consistent increase in the willow flycatcher population was observed, from near absence prior to 1995 (Hubbard 1987) to over 225 nesting territories by 2008 (Ahlers & Moore 2009). Almost 90% of nests were in native vegetation, 10% in mixed stands and only a trace were in tamarisk dominated areas. Both these cases represent numerical expansions of an extant flycatcher populations rather than new colonization, but they provide good evidence that avian populations previously using tamarisk have sufficient resiliency to respond positively to recovery of native-dominated vegetation.

Scale of Restoration There are three levels of restoration or ecosystem recovery to be considered in the context of mitigating potential negative effects of tamarisk biocontrol, each involving a different approach to enhancing reproductive success. The first is to ensure that suitable structural vegetation elements are available in the immediate proximity of currently nesting birds, so that a nest that experiences diminished tamarisk cover will still have residual protection from direct sun physiological stress provided by other species (successful nests exhibited ≥ 90% canopy cover – MacLoed and Koronkiewicz 2009). The second is to promote native vegetation in the general area of known nesting, facilitating the availability of suitable replacement nesting habitat to sustain local viability of returning birds. But the third approach is to restore native vegetation along currently unoccupied (at least by SWFL) river reaches to attract and support the expansion of the population, and ideally to enable de-listing of the species in the future.

Protecting currently nesting birds from exposure by defoliation is probably not a realistic tactic, as it would involve immediate installation of canopy plants at the time of, or just prior to *Diorhabda* colonization and/or defoliation, potentially disrupting behavior of the nesting birds. Alternatively, managers could install artificial shade structures over individual nests if defoliation is imminent. The use of insecticides or chemicals that make the tamarisk foliage unappealing to *Diorhabda* has been discussed and may bear further consideration. Such manipulations, if not necessarily detrimental, are still unlikely to be allowed out of concern for interfering with a listed species. If site fidelity, the tendency to return to prior nesting locations, is high then perhaps woody native plants such as willows could be installed prior to nest establishment, such as the autumn of the previous year. While fidelity to a general location can fairly high, returning birds are actually fairly mobile and are probably not likely to use the same tree or patch (Newell et al. 2003). Vagility is not surprising for a bird associated with highly dynamic fluvial systems where sites can change dramatically between years.

The second tactic of promoting stands of native vegetation in close proximity to known nesting is a reasonable approach to restoration, taking advantage of the same site fidelity behavior but with lesser specificity by occupying alternative plants in the vicinity if they meet the requirements for nest placement. This may be an outcome if the first nesting attempt fails owing to exposure, as the SWFL is documented to regularly re-nest following nest failure (e.g. 25% of females in lower Colorado studies; McLoed and Koronkiewicz 2010). The areal extent of currently occupied vegetation gives a clue as to how large a re-vegetated stand should be to serve this need, and for regional SWFL sites can be fairly narrow and less than a hectare in area (Ellis et al. 2009). Because the dimensional area is not exceptionally large, the expense of moderately intensive site management (manual installation of native plants, irrigation, fencing to exclude herbivores, etc.) can still be a cost-effective approach to protecting endangered species in the short-term. Since SWFL prefer sites adjacent to open water or saturated soils also means, choosing restoration sites based on the availability of water close to the soil surface also means that conditions for growing phreatophytic plants are probably met and further, that irrigation may be a temporary need or even unnecessary. And in some inhabited locations in the Virgin system, nesting stands are comprised of a mixture of *Tamarix* spp. and native plants, mostly *Salix* spp. and some *Populus fremontii*, so restoration efforts would consist of encouraging existing vegetation rather requiring large-scale installation of new plants.

As noted above, such targeted re-vegetation has not been shown previously to attract SWFL in other locations; however, the first clear-cut case of restored site occupation was recently documented in the Virgin River system where the SWFL-tamarisk biocontrol controversy is focused. In the Utah portion of the watershed the Virgin River Program has facilitated mechanical and chemical tamarisk control in proximity to the City of St. George (http://www.virginriverprogram.org/), simultaneous with the introduction and establishment of *D. carinulata* (Bateman et al. 2010). Re-vegetation was implemented by local agencies to restore native vegetation (Mortensen 2008), and by 2009-2010 had developed a dense canopy and multi-layer vegetative structure. The willow flycatcher is known to nest at several sites in this reach of the river, mostly in *Tamarix* despite presence of native willows in the system. Initially there was little change in nest site choice, and in 2009 Utah Division of Wildlife Resources biologists reported 15 nesting attempts by 10 females, almost entirely in tamarisk resulting in 40% nest failures and only two juvenile birds fledged. At least one of the nest trees was defoliated by *D. carinulata,* but a causative role of biocontrol could not be established as the mechanisms for nest failures were not identified; similarly high failure rates are observed elsewhere in the watershed that beetles had not yet colonized, and predation remains the major source of mortality (McLoed and Koronkiewicz 2010).

In 2010, however, there was a substantial behavioral shift to nesting in the restored native vegetation, attendant with a three-fold increase in the number of juveniles fledged (with the caveat that only 9 females were involved in forming territories). This is the best evidence to-date that active restoration of native vegetation can facilitate utilization of such habitat by SWFL, even where tamarisk is still present in the system, and presumably leading to higher reproductive success. A note of caution: major flooding of the Virgin River in December 2010 scoured away a substantial quantity of restored SWFL habitat (S. Meismer, Virgin River Program, pers comm.), so it is unclear how sustainable these efforts will prove to be and illustrates why restoration in flood-prone watersheds must include eco-hydrological evaluation to enhance the probability of long-term success (Stillwater Sciences 2006).

A more fundamental test is to verify whether restoration of native vegetation where the SWFL is no longer present can induce birds to re-occupy these abandoned reaches and river systems. These birds will move fairly large distances, as much as 140 km and between watersheds, although to locations where other conspecifics form territories (Paxton et al. 2007), but it's not inconceivable that they would occupy new areas within the dispersal range if conditions were appropriate. Thus the third, and most robust approach to sustaining and enhancing SWFL populations is to promote watershed-scale restoration of native riparian assemblages. Restoration on the modest scale of sites or stream reaches is capital-intensive (Shafroth et al. 2005; Shafroth et al. 2007), but can still be cost-effective because the objectives are limited in spatial scope with moderately high potential for being achieved. On a larger scale the expense and labor involved in active re-vegetation of many river miles, often where access is limited, would be prohibitive. Our interdisciplinary team has proposed an innovative approach to riparian restoration of the Virgin River ecosystem that could lead to large-scale recovery of native vegetation to the benefit of the SWFL as well as many other riparian-dependent wildlife species, and at the same time may be quite cost-effective.

Propagule Islands for Watershed Restoration Riparian plants that develop taproots from germinating seeds tend to grow more vigorously than transplanted trees (Bell 1998), but natural recruitment depends on availability of adult trees that can produce sufficient seed for extensive dispersal across floodplains, where many if not most sites are not favorable for germination (Mahoney and Rood 1998). Cottonwood trees that can act as seed sources for recruitment are nearly absent from the Arizona/Nevada reaches of the Virgin River, save for limited patches near the towns of Littlefield and Beaver Dam, AZ and Mesquite, NV, and arborescent willows (e.g. *S. gooddingii*) are rare (Fig. I.1.4). Nonetheless, hydrological conditions in the Virgin floodplain should still favor the establishment of these taxa (Mortensen and Weisberg 2010), which are evolved to disperse seeds in synchrony with high flow events (snowmelt run-off or direct precipitation, depending on region) that create the scoured substrates and moisture availability key to their germination and growth (Mahoney and Rood 1998; Stella et al. 2006). Thus, recruitment seems to be limited by the low abundance of adult plants capable of dispersing propagules to extensive reaches, and so recruitment limitation (Soons and Bullock 2008, Lytle and Merritt 2004) could lead to poor replacement by native plants as tamarisk declines in vigor and abundance. Incidentally, unregulated livestock grazing also has an important role in excluding the few native trees that do germinate in the region, and inhibiting maturation of surviving seedlings (M. Taylor and Dudley, unpub. data), but this impact may be lessened if propagule pressure were to be enhanced by expanding the number and distribution seed-bearing adult trees.

A practical approach to promoting extensive seed dispersal and germination is to create a series of protected 'propagule islands', that is, native plant patches that could mature and produce sufficient seed 'rain' or dispersal kernels (Clark et al. 1999) to regenerate a native-dominated riparian woodlands along whole stream reaches under suitable hydrological conditions. This would be particularly valuable, and cost-effective, in the environmental context of *Tamarix* spp. declining in abundance and cover over a very large landscape as biocontrol by *D. carinulata* progresses. Our team is currently evaluating the timing and abundance of seed production and dispersal in sites where mature *P. fremontii* (and *S. gooddingii* to a lesser extent) still occur, with the objective of determining the spatial distribution for propagule island creation that would ensure extensive seed dissemination and post-flood establishment. An eco-hydrological assessment of the floodplain will also be conducted to determine where soil texture and salinity, moisture and seasonal depth to groundwater, and low relative probability of flood scour during high flow events will allow manually installed plants to thrive and achieve maturity. The assessment will also generate predictions of where and to what extent subsequent natural recruitment of native plants is expected to occur. This approach is being applied on a similarly large scale in the San Joaquin River drainage of California by co-operators in the Virgin River project Stella et al. 2006, Stillwater Science 2006) and is related to the concept of defining the 'recruitment box' for favorable riparian plant restoration (particularly cottonwoods) in other western rivers (Rood et al. 2005).

Post-Biocontrol Vegetation of Native Plants and Tamarisk Under this restoration scenario, an acceptable outcome would be composite stands of native willows and cottonwoods interspersed with residual tamarisk. It may be is preferable that soil disturbance be minimized during the course of active re-vegetation, partly to reduce the likelihood that secondary weeds will take advantage of disturbance to dominate the sites (D'Antonio and Myerson 2002; Bay and Sher 2008; Shafroth et al. 2007). In addition, leaving the standing biomass of declining tamarisk in place could afford some degree of physical protection to other plants even as its competitive impact is relaxed, and continue to provide some structural wildlife habitat in the interim. Based on experiences in the upper Virgin watershed, it appears to take roughly two to three years before re-vegetated sites are sufficiently grown in to be re-occupied by SWFL, and this renewal could potentially be accelerated if newly installed plants supplement existing non-native vegetation rather than removing the tamarisk. The goal of weed biocontrol is to suppress, or reduce the dominance of, invasive plants and not to eradicate them, thereby mitigating the negative effects of tamarisk invasion without requiring its total removal. MacLoed and Koronkiewicz (2010) observed that roughly a quarter to three-quarters of cover over SWFL nest sites in this watershed is comprised of *Tamarix* spp. foliage with the rest made up of native vegetation, so best management practices for restoring the habitat in the short term may consist of increasing the proportional composition of native plants (mostly *Salix* spp.) in currently occupied locations. The continued presence of tamarisk could actually enhance the habitat quality of these sites, as well as propagule island restoration sites, because its three-dimensional structure seems useful for encouraging site choice by this and other avian species, and food resources are also enhanced by availability of larval and adult *Diorhabda* (Longland and Dudley 2008).

Over the larger landscape, we similarly see little necessity for the massive reduction of tamarisk biomass, unless the dead and dying material poses a fire hazard or interferes with recovery, where biomass removal, including prescription fire (Brooks et al. 2008), would be recommended. Along the Colorado River near Moab, Utah where agencies have been following tamarisk biocontrol results for five years, there has been an encouraging increase in foliar cover of the shrubby willows, e.g. narrowleaf willow (*Salix exigua*), as existing plants are released from competition following *Tamarix* defoliation (Bean 2007). Unlike the arboreal willows (*S. gooddingii, S. laevigata*, etc.) this clonally spreading species, a.k.a. coyote or sandbar willow, remains common on the Virgin River amongst the dominant *Tamarix* spp. vegetation (Section I, this report) where it could similarly rebound following tamarisk suppression (as it did following mechanical control; Busch and Smith

1995). Shrubby willows comprise a valuable vegetative component in many western locations of the SWFL, providing important mid-level dense foliar cover for shade and refugia from predators (Sogge et al. 2003).

Furthermore, *S. exigua* is more tolerant of fire than are the arboreal taxa (Mount et al. 1996) so can better co-exist interspersed with flammable *Tamarix* spp. vegetation. The risk of wildfire declines as the native proportion of riparian vegetation increases (Drus et al. 2011), and the combination of re-growth of existing plants, re-vegetation with native trees and recruitment of native species into zones where *Tamarix* biocontrol is effective will eventually make these systems more resistant to fire. Absent biological (or mechanical) control treatments, such systems would otherwise likely proceed toward fire-prone tamarisk monocultures (Mortenson and Weisberg 2010). That is why planting native trees is currently a risky management strategy in areas prone to wildfire, such as along the lower Colorado River, so it is possible that tamarisk suppression will facilitate more successful restoration efforts in these regions. Fire has direct effects on endangered wildlife as well, with cases of tamarisk-fueled wildfire destroying active nests of SWFL (Paxton et al. 1996). One of these occurred just recently within the Virgin watershed, in which two active SWFL nests were lost at the Moapa Valley/Warm Springs Natural Area in July 2010 before juvenile birds had fledged (R. Johnson, pers comm); at this fairly small, isolated site re-colonization is uncertain. In the absence of biocontrol to constrain further domination by tamarisk, this will be an increasingly common occurrence throughout the Southwest as wildfire is linked with a feedback loop in which greater tamarisk cover promotes more fire, leading to greater loss of native vegetation, and so on (Drus et al. 2011).

Promoting a substantial portion of native vegetation not only reduces wildfire risk, but is also key to sustaining wildlife as avian abundance in mixed vegetation stands remains relative unchanged until approximately 60-75% dominance by *Tamarix* spp. (van Riper et al. 2008). Food availability may be part of this relationship, as native plants support more herbivorous arthropods than non-native tamarisk (Shafroth et al. 2005), so having a substantial component of native vegetation can mean that more food resources will be available to insectivores than in monocultural systems (c.f. Herrera and Dudley 2003). A native overstory also creates a moderated micro-environment, as *S. gooddingii* canopy maintained cooler and more humid conditions in the SWFL nesting zone (2-4 m height) than other vegetation types (McLoed and Koronkievicz 2010). In the Virgin River ecosystem, the diversity and/or abundance wildlife of several groups, small mammals, reptiles, birds and even bats, is lower in tamarisk monocultures than in mixed species stands, even with a substantial proportion of *Tamarix* spp. present (Bateman et al. 2010; Ostoja et al. unpub data; Kuehn et al. unpub data – Appendices 1 and 2, this report).

In this context the gradual suppression of tamarisk by biocontrol in association with active and passive approaches to riparian restoration may lead to incremental yet sustainable enhancement of this habitat along with associated wildlife species. We know from this and other locations that the SWFL can respond positively to expansion of native vegetation under the appropriate conditions. The regional scope of SWFL population censusing creates the basis for assessing large-scale meta-population dynamics of the sub-species (Lamberson et al. 2000); likewise, a restoration program that combines local actions and regional planning for geographically integrated restoration within and between watersheds, may allow rehabilitation of this and other spatially restricted migratory species through reconnecting meta-population structure and functional movement among suitable patches throughout the Colorado and other southwestern basins.

Restoration and Resolution of Conflicts Between Weed Biocontrol and Endangered Species Protection There is good potential for achieving ecosystem recovery and special status species enhancement in the Virgin River watershed based on a series of initiatives and partnerships focused on restoration of riparian resources to meet water and wildlife conservation goals (Estergard 2008). These are loosely co-ordinated through a multi-agency task force, the Virgin River Conservation Partnership and regional Habitat Conservation Plans and related programs (Clark County MSHCP, Virgin River Habitat Conservation and Recovery Program, Washington County - Virgin River Program, Colorado River Multiple Species Conservation Program). A nascent program will build on these initiatives to facilitate an integrated, watershed-based restoration program targeting SWFL habitat enhancement. The Tamarisk Coalition, a non-governmental organization dedicated to the joint mission of managing tamarisk invasion and promoting riparian restoration, is facilitating a planning effort that involves federal natural resource managers (e.g. Fish and Wildlife Service, Bureau of Reclamation, Geological Survey – Biological Resources Division, Department of Agriculture), state resource agencies in Utah, Arizona and Nevada, university researchers and private consultants to develop and implement a restoration program for the Colorado River Basin to promote native riparian vegetation recovery and habitat provisioning for SWFL in the context of current and anticipated tamarisk biocontrol establishment. Supported by a major private foundation that emphasizes Colorado River conservation issues (Walton Family Foundation), the Virgin River was selected as the first watershed in the Basin for applying habitat enhancement efforts in support of this mission.

By itself, active restoration will not eliminate conflicts over strongly held positions, but it can provide a framework for bringing divergent viewpoints into the same arena. Bridging the conceptual gap between the ‘sides’ of the biocontrol issue will be much easier if a co-operative program can take form that maintains focus on the long-term goal of restoring functioning river systems, not staking out positions regarding the perceptions of risks associated with one of the tools for moving toward that direction. There has been too much speculation in the absence of data regarding the benefits and risks of biocontrol, and the conflict reflects poorly on both the regulatory agencies that have not fully understood the process and expectations of weed biocontrol, and the biocontrol researchers and managers who have not been effective in presenting the case, as well as the cautious and rigorous environment in which modern biocontrol development takes place. Likewise, weed control practitioners and researchers need to broaden their perspectives by recognizing that wildlife concerns, even if ultimately shown to be invalid, are nonetheless real and deserve serious consideration. We have now come to a point where we are literally sitting at the same table, and developing the strategy and tactics for integrating restoration with the on-going *Tamarix* Biocontrol Program. In the course of executing that process, it is anticipated that the points of disagreement may be made more open to challenge, and deliberated through the honest communication that has been lacking in this contentious debate.

The proposed multi-watershed program will involve strategic and rigorous planning to ensure that when executed, restoration will have a high probability of achieving its objectives. A science-based monitoring protocol will be constructed to assess the process of ecosystem recovery, from soil and water dynamics to wildlife habitat use and all the key parameters involved in restoring better function to the system. Information will be used in an adaptive management framework to modify tactics based on data, and applying the approach across watersheds in a cohesive way that can effectively yield replicated exercises in carrying out the program. By incrementally building an objective database on the direct and indirect responses to tamarisk biocontrol, all the participants anticipate the resulting data to form the basis for re-entering negotiations between the Fish and

Wildlife Service and USDA-APHIS, ultimately resolving the regulatory dispute between the agencies and halting the litigation that has held up this and other weed biocontrol programs nationally. Given the moderately recent interest in applying biological control to wildland environments (Newman et al. 1998)…'conservation biocontrol' can and should play a key role in enhancing conservation values and natural resource benefits."

MANAGEMENT RECOMMENDATIONS

Invasive plant control treatments that result in soil disturbance, or damage existing native vegetation, have high potential to promote the establishment or expansion of secondary invasive plants. Repeated follow-up treatments are essential to maintaining control of tamarisk and other invasive plants, and for effectiveness require regular monitoring to ensure that re-treatments take place before weeds reach epidemic populations sizes once again.

The introduction of tamarisk biocontrol to the region, however, fundamentally changes how future weed management should address control issues. Based on results in other riparian systems and on initial observations in the Virgin watershed, traditional control methods are unlikely to be necessary for effecting tamarisk management goals. Complete defoliation multiple times within as season will reduce weed vigor, and suppression should lead to reduced vegetation cover and reproduction, including mortality of some portion of the population and sustained presence at a tolerable density, over the next 3 to 4 years, allowing native woody plants to establish if conditions are suitable.

Traditional weed control treatments have not been effective, to date, in promoting native vegetation that can support native wildlife species, including several that are Covered or considered special status species under the Clark County MSHCP. This is a particular concern because in many cases there is little evidence that native woody species are able to colonize and establish under the conditions created by these methods, and secondary invasion by other weedy species create even greater difficulty for re-establishment of native riparian habitat. A practical approach may involve staggering the tamarisk removal through time and space across the landscape to maintain habitat heterogeneity. To maintain avian community persistence and species diversity in a post-tamarisk-removal environment, it is advisable to include active vegetation restoration as a management prescription. Tamarisk removal by traditional methods alone, in the absence of proper conditions for re-establishment of woody native plants, may not be a desirable approach to riparian management if the objective is to improve habitat for native wildlife. Although it is too early to know the far-reaching effects of tamarisk biocontrol, this non-disruptive technology may provide a preferred alternative for wildlife conservation and enhancement, particularly in combination with active restoration to facilitate recovery of native plants where conditions (soil and hydrology) present difficulties for new establishment. Lack of native seeds, particularly of cottonwood and willows favored by many of the Covered wildlife species, is an important inhibitor of re-establishment of native woodlands, particularly in the presence of widespread, unregulated livestock grazing. We propose a restoration approach that creates a series of protected ‘propagule islands’, fenced and managed native groves that will ultimately produce seeds to facilitate broader native restoration under appropriate hydrological conditions (Dudley and Bean 2011; see Discussion, this report).

Traditional weed control methods have, however, been successful at reducing ‘fuel’ abundance and continuity, thereby significantly reducing fire hazard. Thus, where fuels reduction is the primary management goal, these treatments remain an effective and rapid, albeit expensive, tool for reducing wildfire risk. Again, the presence of tamarisk biocontrol provides new opportunities and challenges for fuels management; challenges, because defoliation and desiccation of tamarisk foliage during ‘fire season’ can modestly increase the risk of ignition and generate public concern over massive ‘browning’ of dominant vegetation near occupied areas. Where feasible, prescribed fire is an appropriate tool for reducing fuels volume, and should be considered where other resources are not at risk. Opportunities are made possible because our data have shown that that the wildfire risk or flammability in tamarisk vegetation, while high when plants are defoliated by the leaf beetle, is only slightly greater than when the same plants are healthy and green. Thus, the incremental reduction in canopy volume owing to repeated herbivory leads to reduced fire intensity and behavior over the course of 2 to 4 years, along with lower probability that associated native trees and woody plants will experience mortality. A goal should be to promote an acceptable mixture of tamarisk and native vegetation, both in cottonwood/willow habitats as well as higher terrace mesquite/acacia habitats, that will sustain wildlife species diversity while reducing the risk of fire ignition and spread, and when fires do occur, they will not lead to type-conversion to tamarisk-dominated and highly fire-prone stands.

The data generated through this and affiliated monitoring and assessment projects that our MSHCP project facilitated should be a basis for future evaluation of whether tamarisk management, including the presence of *Diorhabda carinulata* for biological control, leads to desired enhancement of native riparian vegetation and associated wildlife species in Clark County. The research team that carried out these efforts will continue to explore the feasibility of maintaining a consistent and rigorous approach to ecosystem recovery monitoring and adaptive management of the restoration process, and continue to develop regional partnerships to meet these objectives.

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APPENDIX 1.

Tamarisk vs. Native Plants as Breeding Habitat for Birds on the Virgin River: Evaluating Potential Impacts of Biocontrol on the Avian Community

Background

Historically, riparian areas in the semi-arid southwestern United States provided a mosaic of productive habitats capable of supporting many vertebrate species (Stanford *et al.* 2005, Latterell *et al.* 2006). More than 50 percent of landbirds that breed in the Southwest are directly dependent on riparian habitats, and most other species utilize riparian habitat at some point in their annual cycle (Anderson *et al.* 1977, Knopf *et al.* 1988). Tamarisk and other invasive plants (such as Russian Olive, *Elaeagnus angustifolia*) in the southwestern U.S. are identified as threats to migratory bird populations on nearly all federal, state and local conservation and management plans. Still, numerous bird species have been found to utilize tamarisk during breeding and migration (van Riper et al. 2008), including the federally endangered Southwestern Willow Flycatchers (*Empidonax traillii extimus*) (Sogge *et al.* 2008).

The tamarisk leaf beetle has spread naturally since introduction into most drainages of the upper and mid-Colorado River basin, including release to the Virgin River near St. George, Utah, where Southwestern Willow Flycatchers (hereafter "SWFL") breed in both native and tamarisk habitats and may be exposed to defoliation on their breeding grounds. At their current rate of spread downstream along the Virgin River, tamarisk beetles may expand into the Lower Colorado River in the next few years, where tamarisk is used for breeding by SWFLs and other riparian birds.

Because tamarisk defoliation is viewed by many as a potential risk to nesting birds, it has only begun to appear in the most recent management plans (e.g., Partners in Flight, Nevada Conservation Plan 2010 draft). Research on the impacts of tamarisk beetles on endangered SWFLs is underway in affected systems (e.g., Virgin River; Bateman et al. 2010), but studies on the broader avian community are important and can be beneficial because (1) larger sample sizes can be obtained to provide more reliable information about the mechanisms by which tamarisk beetles impact birds, (2) population-level impacts can be more readily detected and attributed to specific causes for species that are more abundant, and (3) other species of conservation concern, especially those that have adapted to using tamarisk (e.g., Yellow Warbler, *Dendroica petechia*; Yard *et al.*, 2006) may be impacted by beetles, thereby increasing the need to direct management efforts toward them.

There is interest in the impacts of beetle defoliation on avian nesting success because the loss of foliage from nest trees could make nests, and especially those of the SWFL, more visible to predators and increase exposure to the elements (e.g., solar radiation, precipitation), thereby affecting nest temperature and humidity. Studies investigating how nest cover, concealment, and microclimate relate to nesting success, and how defoliation influences these factors, are needed to understand how tamarisk beetles will impact avian reproductive success.

Beetle establishment may also have short-term positive or negative impacts on food resources for birds. If birds readily feed on tamarisk beetles, then the introduction of the beetle, a primary consumer that feeds directly on tamarisk, may provide an abundant food resource, thereby increasing habitat quality for birds, from a resource perspective; conversely, defoliation could limit flowering, and thus pollinators that might otherwise be available as food for insectivores. It is useful to monitor available food via invertebrate surveys, plant phenology via leaf, flowering, and fruiting surveys, and food selection by birds via foraging surveys and crop contents analyses, to properly assess how these factors change with beetle establishment.

In the long-term, pronounced changes in habitat structure and composition as a result of beetle establishment are inevitable. Many systems in the Colorado Basin have broad expanses of monotypic tamarisk stands, while other areas have a mixed tamarisk-native plant composition (van Riper *et al*., 2008). While biocontrol is not expected to eradicate tamarisk, the introduction of beetles to these systems will reduce plant vigor and increase mortality, ultimately leading to a system where tamarisk persists at lower frequencies. The net change in avian productivity in these systems as a result of biocontrol will depend heavily on what plant species are present with and replace tamarisk (Shafroth *et al.* 2005). Understanding how avian diversity and abundance vary in relation to habitat composition prior to beetle establishment (e.g., van Riper *et al.* 2008), will help identify key habitat needs for riparian birds of conservation concern, thereby allowing land managers to devise targeted restoration plans that maximize benefits for these species.

To understand how leaf beetles impact birds and their habitat we must first understand the extent to which birds use tamarisk, and how critical parameters, such as foliar cover, relate to habitat use and reproductive success. In 2009 and 2010 we collected data on habitat associations and nesting success of birds breeding on the Virgin River in Clark County, Nevada and Mojave County, Arizona, prior to the establishment of tamarisk leaf beetles. In general, the goals of this work were to (1) determine how species richness and avian abundance vary among different habitats on the river, and (2) determine how nesting success varies among habitats. In regard to assessing the impacts of leaf beetles, we were particularly interested in understanding (1) which species readily utilize tamarisk habitats, (2) how reproductive success varies between tamarisk habitats versus native habitats, and (3) how foliar cover relates to nesting success.

Methods

Study System The Virgin River is one of the last remaining largely free-flowing rivers in the desert southwest and its lower section, in Clark County, Nevada, has been designated an Important Bird Area (IBA) at the state level by the National Audubon Society. All of the endangered birds in Nevada occur here, as do many of the species identified in the Lowland Riparian section of the Nevada Bird Conservation Plan (Nevada Partners in Flight 2010) and other federal and regional bird conservation lists. Relatively unaltered flow regimes have produced meandering stream channels and extensive riparian habitat along this section of the river, with the potential to hold highly productive bird populations.

As with most river systems in the desert southwest, tamarisk has become a dominant component of the riparian vegetation on the Virgin River, with some estimating it comprises over 80% of the riparian habitat on the lower Virgin River valley (Mortenson and Weisberg 2010). In 2006 tamarisk beetles were introduced to the upper Virgin River watershed near St. George, UT, and they have expanded their range in all directions by approximately 15-20 river km per year since then (Section V, this report). Tamarisk beetles began moving into the study plots in late 2009, after the avian breeding season ended, and affected the two most upstream nest-searching plots during the 2010 breeding season. It is expected that in 2011 the beetles will spread throughout most, if not all, of the remainder of the Virgin River study sites.

Overview of Study Design We used point count surveys to evaluate species richness and avian abundance in riparian habitats along the river, and nest monitoring to assess relationships between nesting success, habitat type and foliar cover. Five replicate study "blocks" were established along the river at Littlefield, Big Bend, Mesquite, Gold Butte and Mormon Mesa. Each block consisted of 4 point count transects (with 6 stations each) and two nest searching plots.

Variable-radius point count surveys were conducted at 120 stations, with equal numbers of stations in each of the following four habitat types: (1) monotypic tamarisk, (2) tamarisk-native "mixed" (tamarisk mixed with cottonwoods, willows and/or mesquite), (3) along the outside edge of the riparian corridor and (4) in areas scourged by the 2005 flood (successional habitats). Survey stations were established at least 250m apart within riparian vegetation, and at least 50m from the edge. Each station was surveyed three times annually, from 15 May to 30 June by at least two different surveyors. Point count surveys lasted 10 min and all species detected by sight or sound were recorded, along with their distance from the point, mode of detection (e.g., visual, song, call, etc), sex, and age.

Nest monitoring took place from 1 April-15 July annually on monotypic tamarisk (N=5) and tamarisk-native "mixed" (N=5) plots, following BBIRD nest monitoring protocols (Martin *et al.* 1997). Monotypic tamarisk plots were comprised of at least 90% tamarisk trees, while mixed plots consisted of tamarisk trees mixed with a variable native component (20-100% native trees; e.g., willows, cottonwoods, mesquite). A single plot was comprised of two 100m radius areas around pairs of adjacent point count stations (two 100m radius circles = 6.3 ha total). We monitored all nests found, but focused on searching for nests for the following species: Yellow Warbler (*Dendroica petechia*), Yellow-breasted Chat (*Icteria virens*), Song Sparrow (*Melospiza melodia*), Blue-gray Gnatcatcher (*Polioptila caerulea*), Arizona Bell's Vireo (*Vireo bellii arizonae*), Lucy's Warbler (*Vermivora luciae*), Abert's Towhee (*Pipilo aberti*) and Verdin (*Auriparus flaviceps*). Once found, nests were checked every 2-5 days to determine if they remained active or had failed (e.g., due to depredation).

Within one week after each nest finished (fledged young or failed), we assessed foliar cover using a light meter to measure the amount of solar radiation (photosynthetically active radiation, or "PAR"; 400-700nm) penetrating through the foliage at 9 points (1 directly over nest, and 2 in each of the cardinal directions) within 1 m of the nest. As a reference, we also recorded ambient light levels (measured in the open sunlight). At the end of the breeding season (mid-late July) vegetation at all nest sites was characterized following BBIRD protocols (Martin *et al.* 1997). Briefly, all woody plants were categorized and counted by species and size class (dbh) within an 11.3 m radius of the nest site. Estimates of canopy composition and height were also made for the 11.3 m plot.

Data Analysis Point count data analyses were conducted using SPSS 12.0.1 (SPSS Inc.). To minimize the potential for detection probability biases in the analysis of point count data, only detections that occurred within 50m of the survey station were included all analyses. Each station was surveyed six times during 2009-2010, and for any one species, we used the maximum number of individuals of that species detected on a single visit as the final estimate for that species' abundance at a station. Individuals detected flying high over the survey station were also excluded from the analysis, as these species were assumed not to be using the specific habitat type present.

Nest success data were analyzed using a modification of the Mayfield method (Mayfield 1961, Manolis et al. 2000), which yields a daily probability of nest failure by dividing the number of nests observed to fail by the total number of days over which the nests were observed while active. For each nest we calculated the number of days of observation, beginning with the day the nest was found and extending through the midpoint between the last visit the nest was observed active, and the first visit the nest was observed inactive. Daily failure rates were compared between groups of nests by calculating 95% confidence intervals following methods outlined by Johnson (1979).

Foliar cover was defined as the percentage of ambient sunlight prevented from reaching through the canopy to the plane of the nest and was calculated by dividing the average of all 9 light meter measurements made at the nests by the control reading taken in open sunlight, then multiplying by 100 and subtracting the product from 100. Thus, values range from 0-100, and higher values indicate greater amounts of foliar cover.

Nest-site vegetation was assessed using data from vegetation surveys, in which we calculated the percentage of woody stems that were tamarisk, versus non-tamarisk, for the 11.3 m radius area around the nest. Stems were classified into the following size-classes (according to their diameter at breast height): <2.5 cm, 2.5-8 cm, 8-23 cm, 23-38 cm and >38 cm. For each nest, the number of stems in each size-class was multiplied by the mean diameter for that class to create a stem "area" index, and totals were calculated for tamarisk and non-tamarisk stems. The percent tamarisk at the nest site was then calculated as the total stem area for tamarisk, divided by the total stem area for tamarisk and non-tamarisk plants combined.

Results

Avian Diversity and Habitat Associations Surveyors detected 92 species of birds while traversing transects and conducting point count surveys. However, the total number of bird species detected within 50m of stations and during actual surveys was 74. Avian diversity (Figure A1.1a) and abundance (Figure A1.1b) both varied among the four habitat types surveyed, with tamarisk-native mixed habitats having the highest mean diversity, at 17.4 +/- 0.64 species per station, and successional habitat having the lowest mean diversity, at 13.37 +/- 0.58 species per station. Though successional habitats had low diversity on a *per station* basis, the total number of species detected during point count surveys in mixed versus successional habitats was similar, at 55 and 54 species, respectively. Mixed habitats also had the highest densities of birds by a large margin, at 29.4 +/- 1.19 individuals per station, versus the other three habitat types which ranged only from 19.90-21.67 individuals per station.

Lucy's Warblers and Abert's Towhees were atop the list of the 10 most frequently detected species (Table A1.1). The brood parasitic Brown-headed Cowbird (*Molothrus ater*), which lays its eggs in the nests of many riparian birds and reduces their nesting success, was the 6th most frequently detected bird species overall. A look at the relative abundances of 11 species in mixed versus tamarisk habitats (Figure A1.2) revealed that all but four species showed a distinct preference for mixed habitats. The four species that did not show this preference included the Song Sparrow, Abert's Towhee, Bewick's Wren and Lucy's Warbler. Lucy's Warblers and Bewick's Wrens may find tamarisk habitats suitable because both species prefer to build their nests in cavity-like environments. Our observations showed that they often built their nests in the clumps of dead tamarisk leaves that accumulate in branches. Abert's Towhees and Song Sparrows are both omnivorous species, while the remaining birds that clearly prefer mixed habitats are all insectivorous. In fact, when all birds detected during surveys were classified as either Insectivorous, omnivorous, granivorous or carnivorous, the only one to show a significantly different abundance in mixed versus tamarisk habitats were insectivorous bird species (Figure A1.3).

Nesting Success

Overall, nests were observed for a total of 2,798.5 days, on which failure was observed on 75 days, for a daily probability of nest failure of 2.68% (CI = 2.07-3.29%). For a typical riparian bird species, this translates into a 49.3% chance of successfully surviving the entire nesting cycle of 26 days. The two most common causes of nest failure were depredation (all nest contents disappeared before the young could have fledged successfully; 61.9% of failures), and brood parasitism by Brown-headed Cowbirds (30.1%). The only other cause of failure was nest abandonment for unknown reasons (8%).

Both the daily probability of nest failure (Figure 4a) and the overall probability of cowbird parasitism (Figure 4b) varied significantly depending on the percent tamarisk at nest sites. Nest sites comprised of 0-25% tamarisk stems had higher daily rates of failure (6.1 +/- 1.4%), and parasitism rates (60%) compared to nests in any of the categories containing 25-100 % tamarisk. However, because these results include data from nests for all (focal) species and for all nest plots combined, this difference could be due to two potentially confounding factors. First, it could be that certain nest monitoring plots (e.g., mixed plots, where there is little tamarisk), have a greater ambient risk of nest predation and parasitism. Second, it could be that certain species, which prefer relatively tamarisk-free sites, are prone to higher nest predation and parasitism. To address the first possibility, we compared nesting success and cowbird parasitism rates between nest sites that were dominated (>50 stem area) by native plants, versus nest sites dominated (>50%) by tamarisk stems (Figures A1.5a and A1.5b), at the six plots from which we collected the most nesting data. Four of these plots were mixed plots and in each case, birds that used nest sites dominated by tamarisk had lower nest failure and lower cowbird parasitism rates than birds nesting in sites dominated by native plants. We then looked at the three species for which we had monitored the greatest number of nests and compared their nest failure rates and cowbird parasitism rates between native-dominated and tamarisk-dominated nest sites (Figures A1.6a and A1.6b). In this case, Yellow Warblers and Bell's Vireos had lower failure and parasitism rates in tamarisk dominated sites, while the Yellow-breasted Chat showed the opposite pattern for nest failure (but not cowbird parasitism). Overall, these results suggest that the effect of tamarisk dominance on nesting success must be evaluated on a species-specific basis, but that at least some species have higher success nesting in tamarisk compared to native-dominated nest sites.

Among focal species, there was considerable variation in the amount of foliar cover above nests (Figure A1.7), with species-specific averages ranging from 36.3% for Black-tailed Gnatcatchers to 89.8% for Song Sparrows. For the seven focal species, mean foliar cover values did not correlate with the mean daily probability of nest failure (Pearson correlation coefficient = -0.319; P=0.441) or the probability of cowbird parasitism (Pearson correlation coefficient = 0.085; P=0.842). Because the variation in foliar cover is so great between species it is possible that each species adopts its own optimal level of cover and that individuals that stray from this mean value may be at greater risk of predation or parasitism. To test this, we calculated a residual foliar cover value (difference between species-specific mean value, and observed value) for each nest. For each species, we then classified all nests into four categories, according to the quartiles they fell within among all nests: (1) very low cover, (2) low cover, (3) high cover and (4) very high cover. We found that nests with extreme residual foliar cover scores (very low or very high cover), had the highest daily probability of nest failure (Figure 8a) and the highest probabilities of cowbird parasitism (Figure A1.8b).

Discussion

Our results indicate that birds are less diverse and abundant in monotypic tamarisk habitat, particularly when compared with native-tamarisk mixed habitat. Among the species that are found in both habitat types, the majority show a clear preference for native habitats. Species diet is correlated with this preference, in that insectivorous species are relatively less abundant in tamarisk habitats compared to species with more diverse diets. Other studies have reported a lower abundance of invertebrate food resources in tamarisk habitats, compared to native habitats in the desert southwest (Durst *et al.* 2008) and this may explain the patterns observed on the Virgin River. In regard to the potential impacts of biocontrol on birds, the introduction of leaf beetles to this system may provide an important invertebrate food resource in tamarisk habitats and it will be interesting to see how the avian community responds to this, particularly the insectivorous birds. The lack of detailed vegetation data for our point count stations precludes us from investigating relationships between avian diversity and abundance, and varied levels of tamarisk when mixed with native plants. By estimation, our “mixed” point count stations had tamarisk components that ranged from 0-70%. One study has found that the highest levels of avian diversity occur when tamarisk is present at moderate levels (van Riper et al. 2008), and it would be informative to understand how the avian community metrics on the Virgin River vary through the observed range of mixed habitats.

Though monotypic tamarisk habitat held lower avian diversity and abundance than mixed habitat, there are still a number of bird species that use tamarisk regularly during the breeding season. Our results indicate that tamarisk is not lower quality nesting habitat than native habitat for a number of species, as nest failure and cowbird parasitism rates actually declined with an increasing prevalence of tamarisk at nest sites. If defoliation of nest sites in tamarisk is found to negatively impact avian nesting success in the short-term, the impact on the avian community of the Virgin River may depend on whether birds are capable of responding behaviorally; for example, by choosing nest sites that can remain well-concealed after a defoliation event, or by seeking out native plants within mixed tamarisk-native nest sites, in which to construct nests.

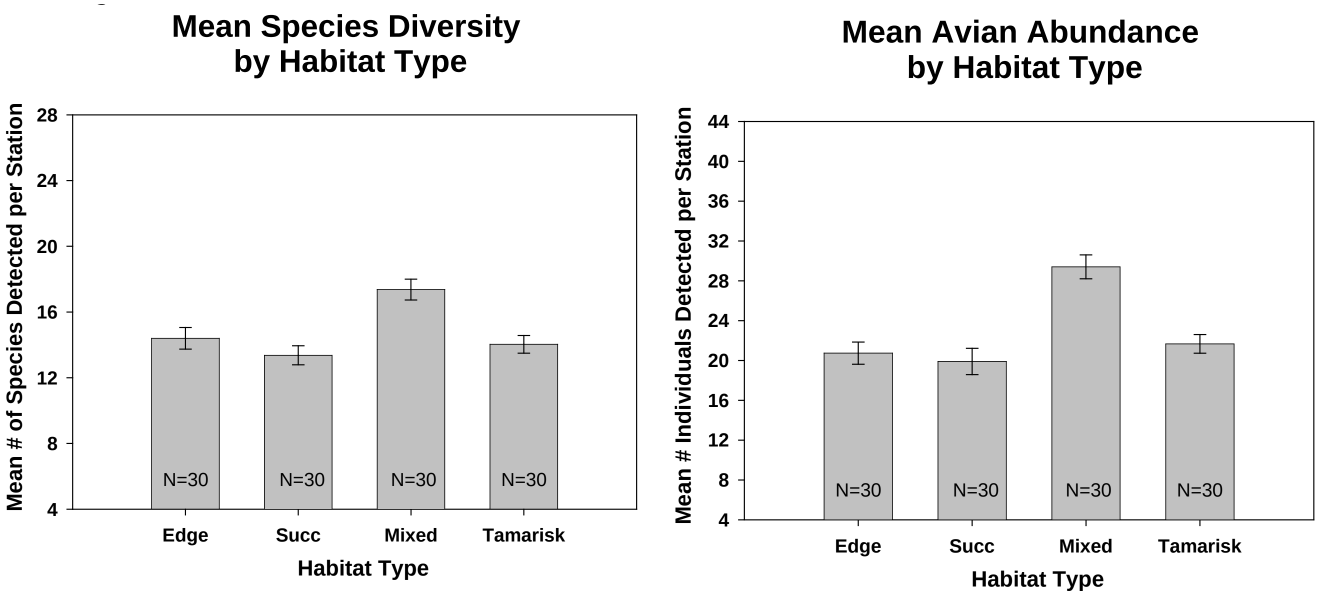
More than 80% of habitat in the lower Virgin Valley is tamarisk-dominant. However, the value of even a small native plant component within tamarisk stands should be evaluated in terms of its potential to offer refuge. If leaf beetles ultimately cause high rates of tamarisk mortality, the potential long-term negative impacts of biocontrol on the avian community could be large, unless natural native plant recruitment occurs or restoration is in place to ensure the replacement of riparian habitat in the wake of tamarisk mortality. This will of course depend on the rate of mortality incurred by tamarisk plants as a result of herbivory by leaf beetles, and how birds respond to this mortality.

If tamarisk mortality is low, or takes many years to occur, bird species that nest within tamarisk will be subject to the potential for defoliation of nest sites each season. If defoliation during the nesting cycle reduces nesting success, the bird species that will be at the greatest risk of population declines within the Virgin River system in this regard are those that (1) prefer tamarisk habitat over native (or are at least abundant in tamarisk habitats) and (2) have high foliar cover requirements. The species that fit both of these criteria include the Song Sparrow, Abert’s Towhee, Yellow-breasted Chat and Yellow Warbler. Though Bell’s Vireos have high nest cover, they are relatively uncommon in tamarisk habitat. Species that may be particularly resilient to the effects of defoliation include those that nest in cavities, or with at least some structure over the top of their nests, as this may allow nests to remain concealed and protected from direct sunlight even through defoliation events. These species include the Bewick’s Wren, Lucy’s Warbler and Verdin; with the latter species building completely domed nests. It should be noted that although we found no relationship between mean foliar cover and nest failure when compared across our seven focal species, defoliation will dramatically reduce foliar cover at nest sites and produce values much lower than those normally observed. This relationship will thus require further investigation after the arrival of leaf beetles at study sites.

A final factor that will affect how the different bird species are affected by defoliation is the timing of the nesting season. Nearly all bird species that breed on the Virgin River initiate their nesting before the onset of widespread defoliation by beetles and their larvae in late May, and cease nesting by early July. The single exception is the SWFL, which begins breeding during mid-May, just before the defoliation period begins. It is thus likely that many species will evade the effects of defoliation on at least one nesting attempt during the season, and may benefit from the abundance of larvae as they emerge from egg cases, but have not fully defoliated nest trees. Defoliation is also spatially heterogeneous during late May and June, meaning that individuals in certain areas each season may be able to completely escape defoliation.

Citations (citations to Appendix 1 are included in general References section)

Figure A1.1. Avian diversity (a) and abundance (b) (+/- 1 SE) among four key habitat types.



a. b.

Figure A1.2. Relative abundances (+/_ 1 SE) of 11 bird species in monotypic Tamarisk versus native-tamarisk Mixed habitat

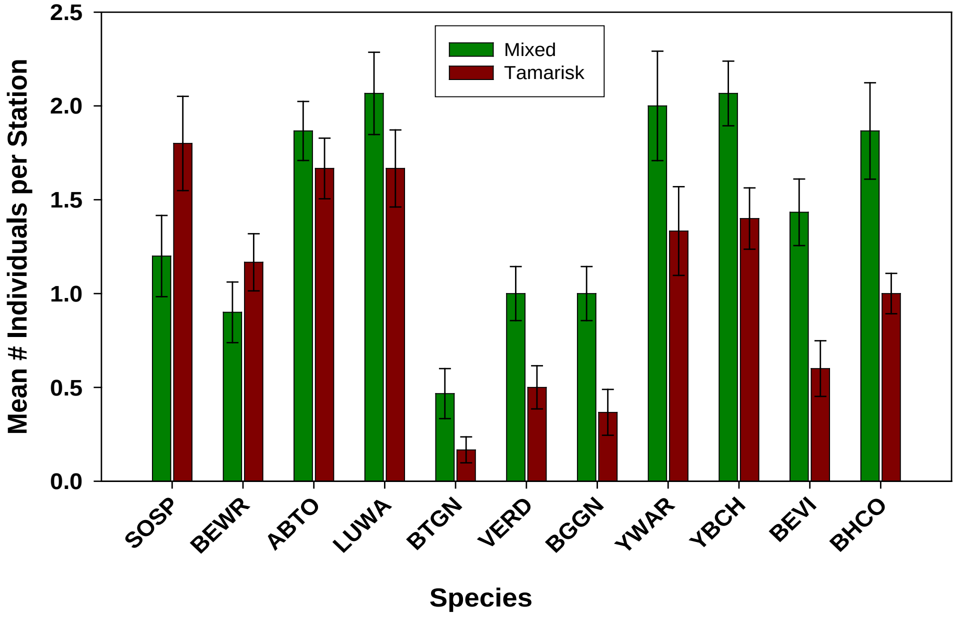


Figure A1.3: Mean abundance (+/- 1 SE) of insectivorous, omnivorous, granivorous and carnivorous birds in monotypic Tamarisk versus native-tamarisk Mixed.

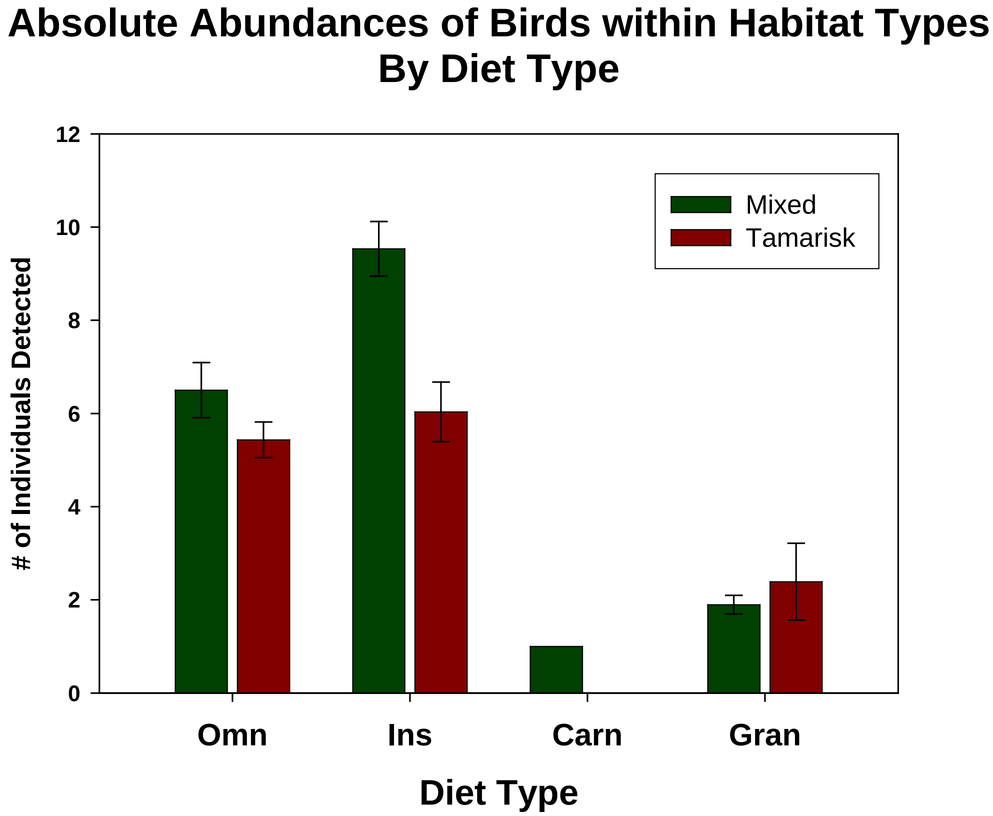


Figure A1.4. Daily probability (+/- 1 SE) of nest failure (a) and probability of cowbird parasitism (b) at nests belonging to 4 categories of tamarisk stem density (0-25%, 26-50%, 51-75% and 76-100%).

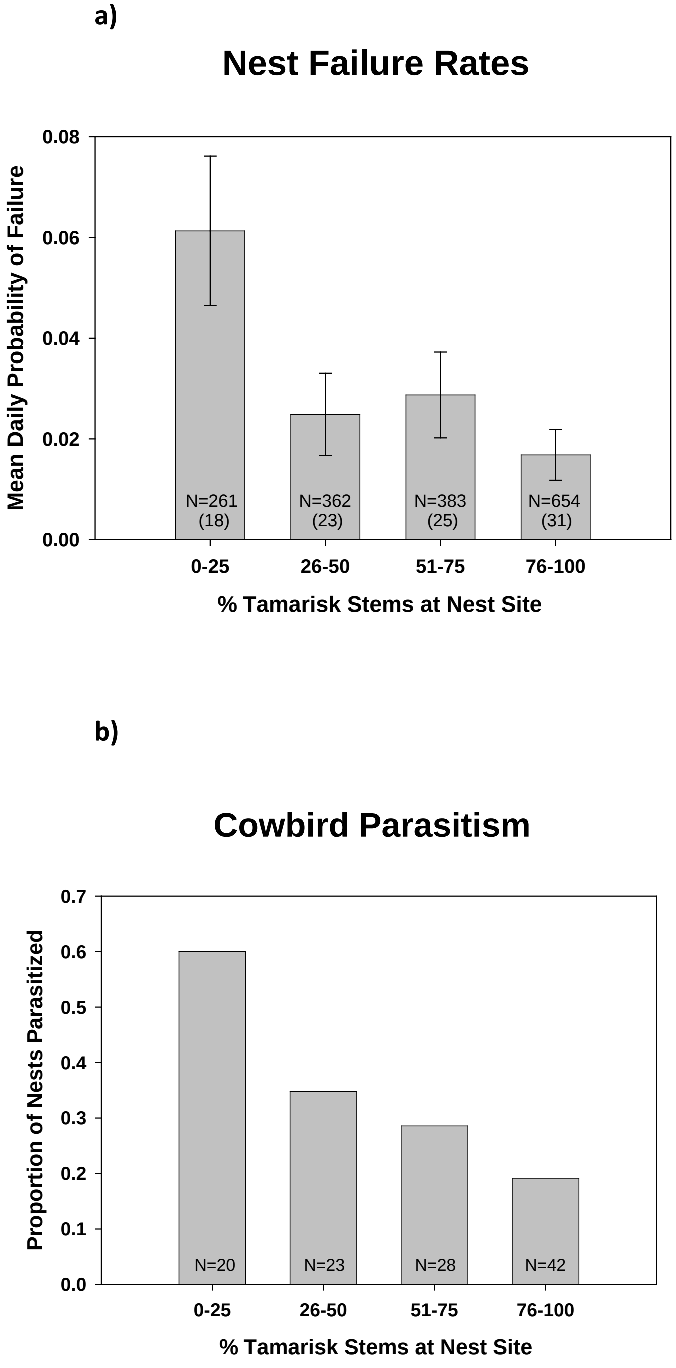


Figure A1.5. Daily probability (+/- a SE) of nest failure (a) and probability of cowbird parasitism (b) at nests found on 6 nest searching plots. Nest sites dominated by tamarisk are compared with nest sites dominated with native plants.

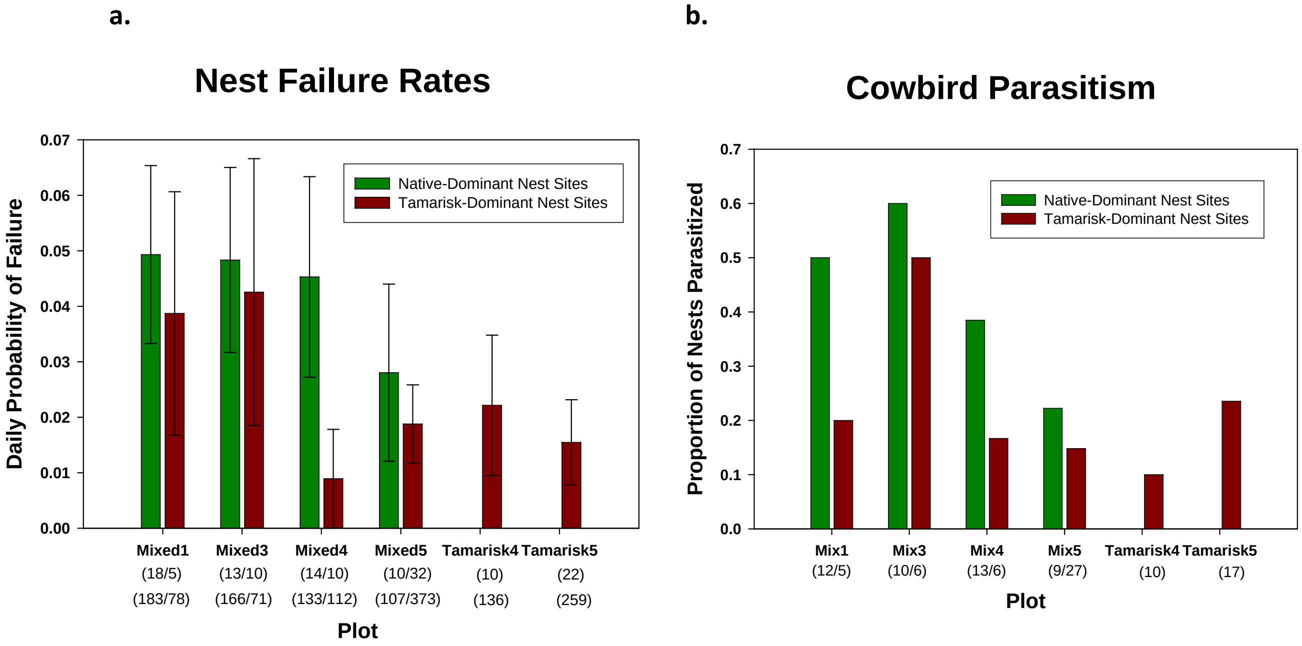


Figure A1.6. Daily probability (+/- a SE) of nest failure (a) and probability of cowbird parasitism (b) at nests of three focal species. Nest sites dominated by tamarisk are compared with nest sites dominated with native plants.

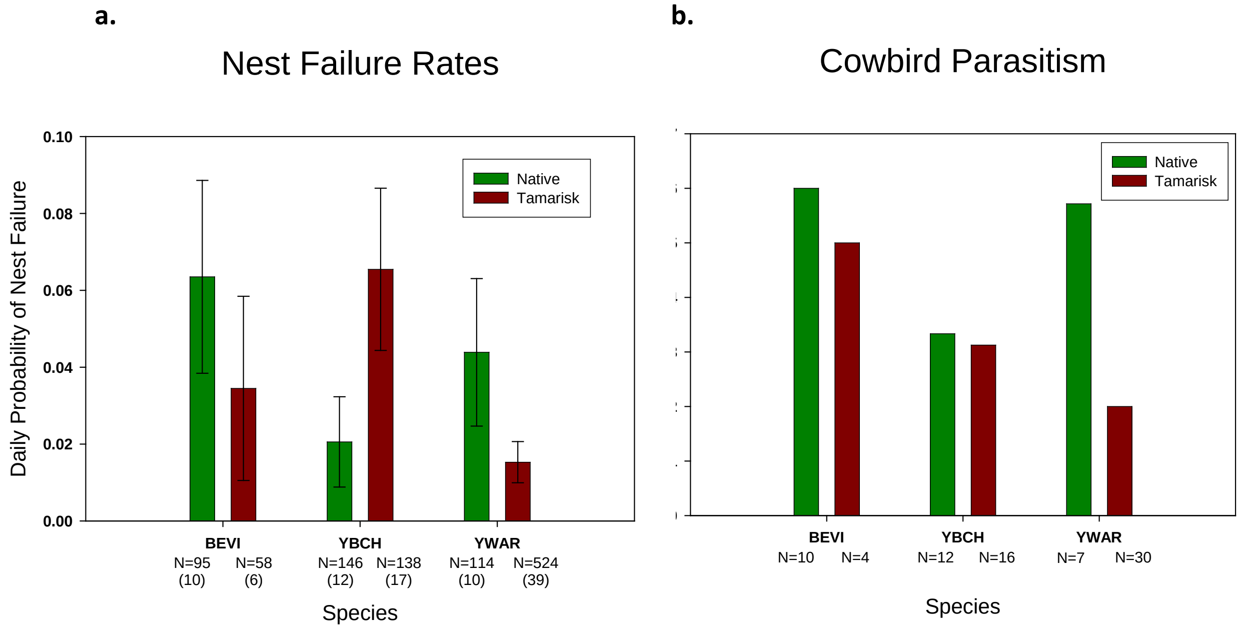


Figure A1.7. Mean (+/- 1 SE) of foliar cover (% of ambient light prevented from penetrating to nest) at nests sites for focal species

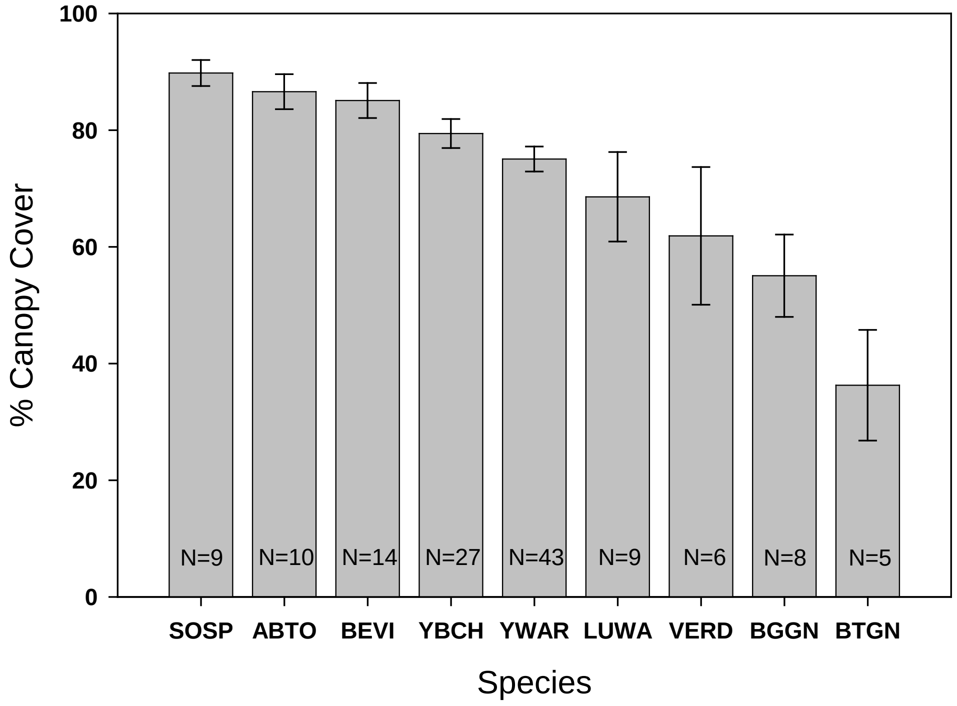


Figure A1.8. Daily probability (+/- 1 SE) of nest failure (a) and probability of cowbird parasitism (b) at nests of all focal species combined, as a function of the quartile in which their residual foliar cover value belongs (see text).

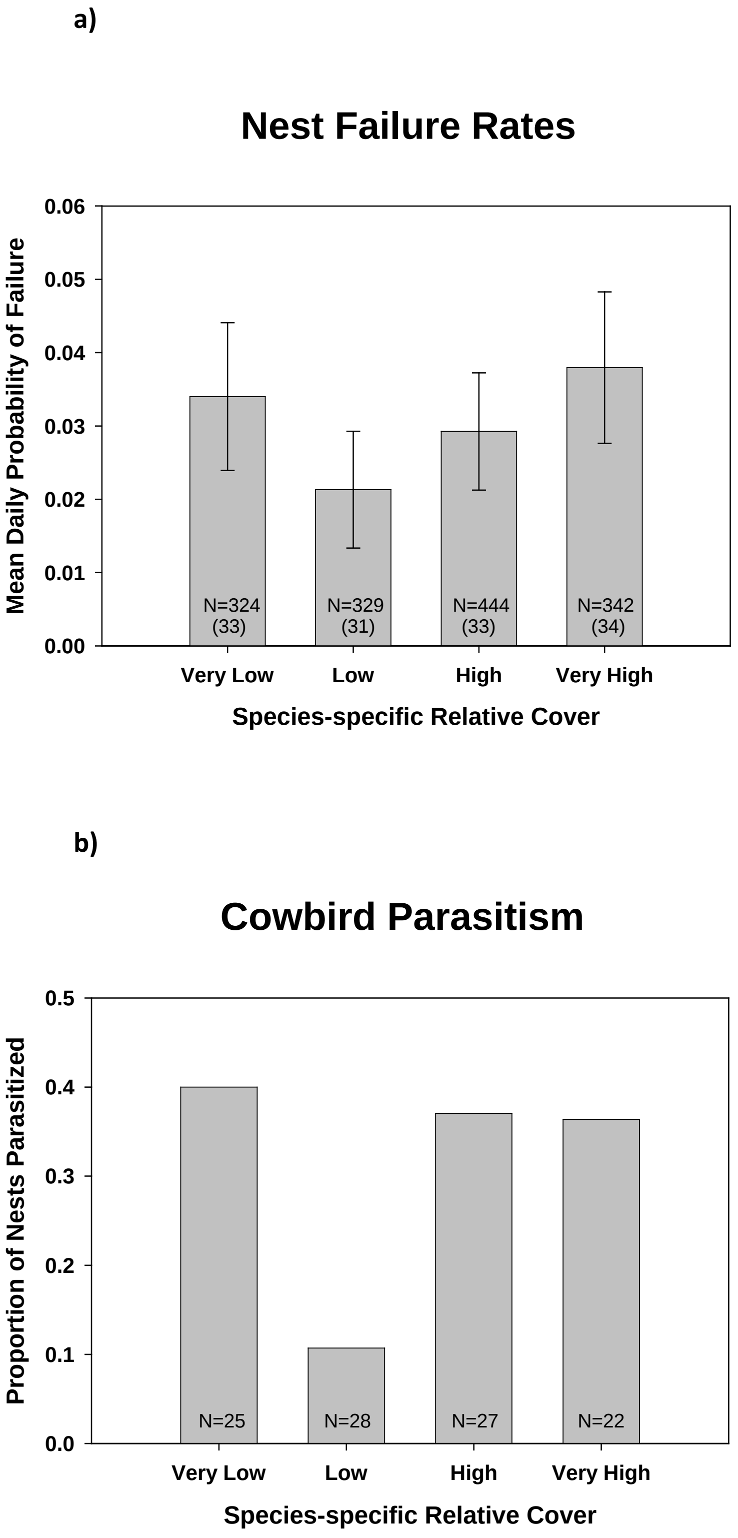


Table A1.1. The ten most abundant species (mean # of individuals per station) detected in point counts conducted at all point count stations combined.

Species	**# per Station**
LUWA	**1.700**
ABTO	**1.475**
NRWS	**1.442**
MODO	**1.392**
BHCO	**1.333**
YBCH	**1.300**
YWAR	**1.300**
SOSP	**1.058**
GAQU	**0.917**

APPENDIX 2. Chiroptera (Bats) Associated with Vegetation Types in the Virgin River Floodplain, and Implications of Tamarisk Biocontrol

Introduction

In 2006, bio-control tamarisk leaf beetles (*Diorhabda spp*) were released on the Virgin River near Saint George, Utah to control invasive tamarisk (*Tamarix spp*). The beetles defoliate tamarisk vegetation and significantly alter the habitat. The purpose of this preliminary study was to collect data on species presence, habitat associations, and activity along the Virgin River before and after beetle establishment. By the start of the study, three of five sampled sites had fully established beetle populations.

Methods

The Anabat digital sonogram method of assessing bat presence was used in several vegetation types to determine if there were differences in species utilization and abundance among the habitat types most common in the Virgin floodplain. The approach and conditions are below.

Anabat Placement

- Anabats (Model SD2) were placed 400 or more meters apart
- Bunkerfield was an exception-habitats exist closer together-sites were ~250m apart
- Anabats collected data for 10.5 hours/night
- Each habitat was surveyed for 8 or more days
- Sites averaged 17.5 km apart, minimum distance of 8.3 km

Habitat Selection

- Habitat types surveyed: Cottonwood/Willow, Marsh, Mesquite, Tamarisk Dry, Tamarisk Wet, Burned
- Habitat sites required 50% or more of specified vegetation for 100m distance
- Tamarisk soil moisture was use to determine dry vs. wet habitat
- Burned sites were 85% or more burned tamarisk snags

Call Identification

When possible, calls were identified to species, otherwise calls were grouped and assumed to be present in habitat for the following:

- *Eptesicus fuscus ,Tadarida brasiliensis*, *Antrozous pallidus*, and *Lasionycteris noctivagas*
- *Myotis yumanensis and Myotis californicus*
- *Lasiurus blossevillii and Parastrellus hesperus*

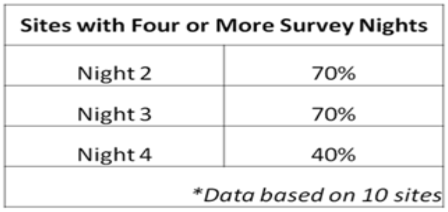
In this preliminary assessment of bats present at the Virgin River, the fact that additional species continued to be detected each successive night of sampling (Table 2) indicated that species richness was likely to be even greater than the results presented. Future surveys will be continued for a longer period to ensure that an asymptotic plateau is achieved that would suggest that we have the full complement of bat species detected during the survey period.

Although species richness was fairly high in each of the vegetation types sampled (Fig. 1), there was a tendency to find the highest richness in native-dominated sampling sites. Furthermore, ‘wet’ sites produced greater detections of species than did drier sites, perhaps indicating that bats were able to secure greater food resources of insects in sites with higher moisture. The ‘burned’ sites were the lowest producers of detected species, presumably because these sites were both drier than other sampling areas, and because they were tamarisk-dominated prior to burns occurring. The greater species richness was also indicated when ‘native’ and ‘non-native’ vegetation types were each aggregated, showing more clearly that tamarisk is less attractive to foraging bat species than are vegetation types with a substantial native component (Fig. 2). This was somewhat surprising because we had not anticipated that the type of plant would partially determine the composition of bats foraging in those areas, but does suggest that further studies should examine the linkage between vegetation type and food resources (insects) available in and above the canopy of these habitats.

Table A2.1. Presence of bat species determined from sonagram analysis for habitat types. Habitat Symbols are: CW = Cottonwood/Willow; MA = Marsh; ME = Mesquite Bosque; TD = Tamarisk (Dry); TW = Tamarisk (Wet); BU = Burned Tamarisk

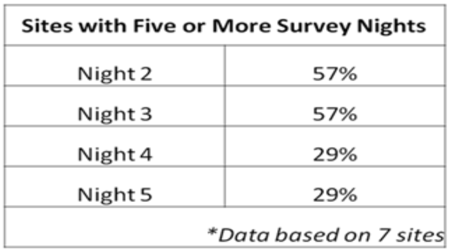
Bat Species		**Detected in Habitat Type**
Big Brown Bat	*Eptesicus fuscus*	CW, MA, ME, TD, TW, BU
Big Freetailed Bat	*Nyctinomops macrotis*	CW, ME
California Leaf-nosed Bat	*Macrotus californicus*	CW, MA, ME, TD, TW
California Myotis	*Myotis californicus*	CW, MA, ME, TD, TW, BU
Hoary Bat	*Lasiurus cinereus*	CW, ME
Lappet-browed Bat	*Idionycteris phyllotis*	CW
Mexican Freetailed Bat	*Tadarida brasiliensis*	CW, MA, ME, TD, TW, BU
Pallid Bat	*Antrozous pallidus*	CW, MA, ME, TD, TW, BU
Pocketed Freetailed Bat	*Nyctinomops femorosaccus*	BU
Silver-haired Bat	*Lasionycteris noctivagans*	CW, MA, ME, TD, TW
Spotted Bat	*Euderma maculatum*	CW
Townsend's Big-eared Bat	*Corynorhinus townsendii*	ME, TW
Fringed Myotis	*Myotis thysanodes*	ME, TD
Western Mastiff Bat	*Eumops perotis*	CW, ME
Canyon Bat	*Parastrellus hesperus*	CW, MA, ME, TD, TW, BU
Western Red Bat	*Lasiurus blossevillii*	CW, MA, ME, TD, TW, BU
Western Yellow Bat	*Lasiurus xanthinus*	CW, MA, ME, TD, TW
Yuma Myotis	*Myotis yumanensis*	CW, MA, ME, TD, TW, BU

Table A2.2. Unique detections following the first night of vocalization sampling.



Sites with Four or More Survey Nights	
Night 2	70%
Night 3	70%
Night 4	40%
	**Data based on 10 sites*

Comparing 10 surveys, representing every site and habitat type, we determined that 40% of survey sites still detected new species on night 4.



Sites with Five or More Survey Nights	
Night 2	57%
Night 3	57%
Night 4	29%
Night 5	29%
	**Data based on 7 sites*

Comparing 7 surveys, representing every site and habitat type, we determined that 29% of survey sites still detected new species on night 5.

Figure A1.1. Mean values for species detected per vegetation type over the course of 5 sampling nights.

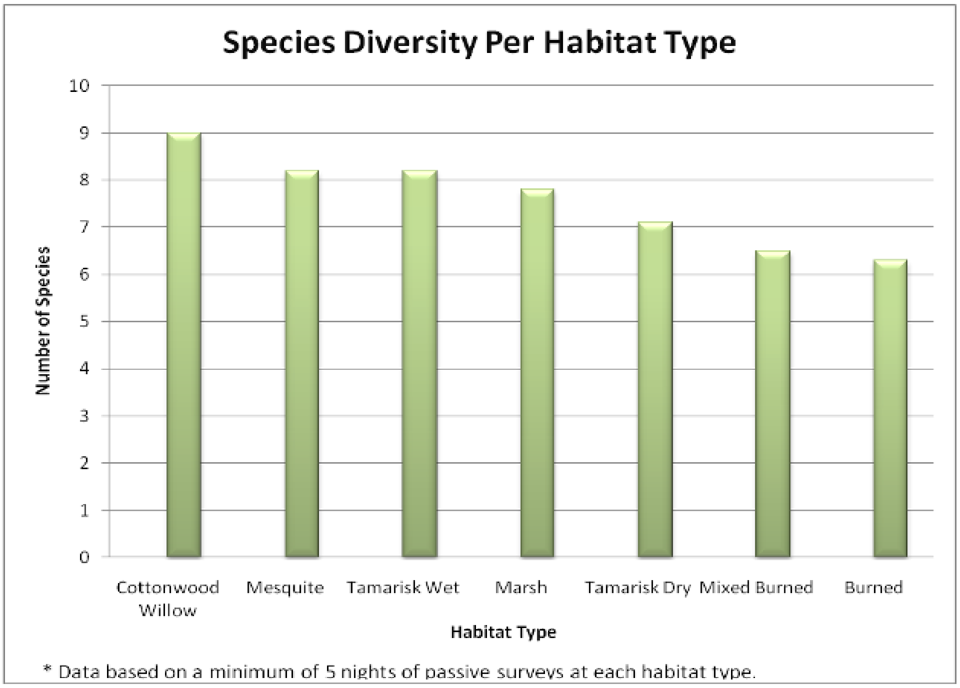
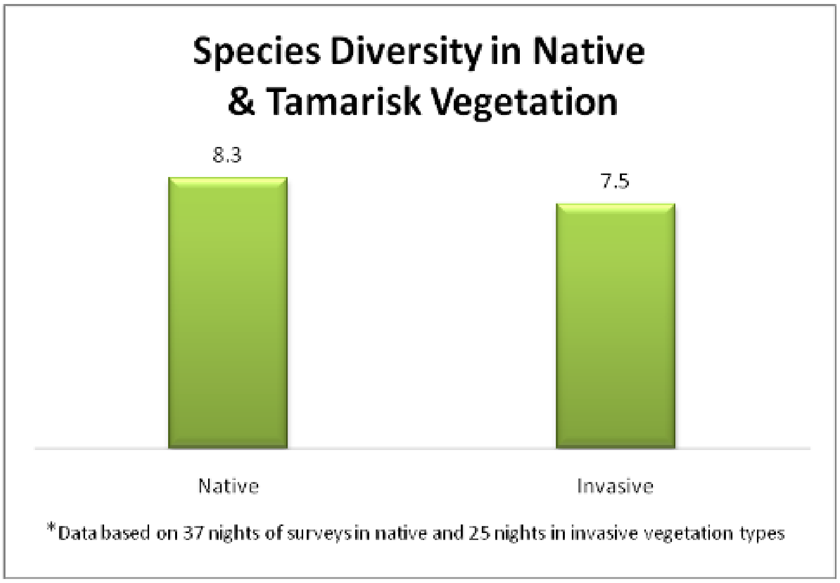


Figure A2.2. Mean number of species associated with combined native vegetation (Cottonwood, Marsh & Mesquite types) and non-native tamarisk (Tamarisk & Wet tamarisk & Dry); burned site not included in analysis. Error estimates not given owing to preliminary nature of these data.



APPENDIX 3. Effects of Tamarisk Biocontrol on Evapotranspiration and Water Resources

Background

Tamarix ramossisima, the most common species of tamarisk in the lower portion of the Virgin River, is a facultative phreatophyte and therefore may be accessing both ground and river water along with vadose zone water. Tamarisk commonly occurs in dense thickets which can lead to high water evapotranspiration (ET) rates, much of which emanates from relatively shallow groundwater. Extraction of groundwater can reduce stream flow and increase the salinity of soil, leading to less available water, and potentially degrading water quality, for irrigation and potable uses. The risk is especially apparent for many western states, such as Nevada, which depend on rivers that are already recording reduced stream flow due to drought and earlier melting of winter snowpack (Barnett and Pierce 2008).

The implementation of biological control using the tamarisk leaf beetle, *Diorhabda carinulata* has already led to large-scale establishment along the upper Colorado River basin and the Virgin River tributary, which drains portions of Utah, Arizona, and Nevada before it empties into Lake Mead. Currently, beetle-caused defoliation has progressed by 2010 to the Riverside Bridge near Riverside, NV by the end of 2010 (32 km), and beetles are present and feeding as of early June 2011 about 20 km below this location but defoliation is not yet apparent at this early date.

The relatively rapid progression of these beetles down the Colorado River basin provides a unique opportunity to evaluate changes in ET during the course of beetle migration and colonization down the watershed. The primary goal of the research was, thus, to estimate evapotranspiration (ET) prior to and following episodic herbivory by the leaf beetle, on a dense stand of tamarisk located at Meadowland's Farm, approximately 2 km from Riverside Bridge, NV, and another ET station with ET tower in the Mormon Mesa portion of the River, 5 km upstream of Lake Mead. In the spring of 2010 an eddy covariance (EC) station was installed at Meadowland's farm to quantify the potential change in ET. In particular, the research goals for this project are to:

1. *Quantify ET prior to and following episodic herbivory by D. carinulata*: It is critical to the understanding of any potential water savings through tamarisk defoliation along the Virgin River, to quantify ET prior to and following episodic herbivory.

2. *Calculate the difference between ET prior to and following herbivory which may contribute to a net savings of water along the Virgin River*: By quantifying ET prior to and following episodic herbivory (objective 1) we can then calculate a difference. Although this difference will not directly correspond to a net water savings (due to other vegetation using the saved water), it will give insight into potential water savings for both native vegetation use and recharge into the Virgin River.

3. *Monitor stream flow and daily groundwater oscillations from groundwater wells*: Stream flow and daily groundwater oscillations at several locations will be monitored and an attempt will be made to correlate with ET (both prior to and following episodic herbivory). In many systems a strong groundwater oscillation can be observed as a direct effect of daily groundwater use by the vegetation. These oscillations typically become stronger later in the growing season as recharge becomes less, vadose zone soil moisture becomes depleted and tamarisk relies more heavily on groundwater sources.

The 2010 data for ET, groundwater, and streamflow have all been collected, and will be an essential component for quantifying potential water savings from bio-control following *D. carinulata* arrival and defoliation in 2011.

METHODS

In late February an EC station (Campbell Scientific, Logan, UT) was installed at Meadowland's Farm, near Riverside, NV.

Task 1: Quantify ET prior to and following episodic herbivory by *D. carinulata*

Figure A3.1. Photo of Eddy Covariance set up in February of 2010.

The approach for estimating ET from the EC station is using an energy balance:

$$\lambda E = Rn - G - H \quad (1)$$

where λE is the latent heat of vaporization, Rn is net radiation, G is soil heat flux, and H is sensible heat. The EC system (Fig. 3) is mounted on a 5-m galvanized steel tripod, which includes a 3D sonic anemometer (model CSAT3) mounted one meter above the canopy, an open-path infrared gas analyzer (model CS7500) mounted one meter above the canopy, a REBS net radiometer (model Q7.1), two soil heat flux plates (model HFP01SC), two soil thermocouple probes (model TCAV-L), two soil water reflectometers (model CS616), and a combination air temperature/relative humidity probe (model HMP45C-L).

Data are stored on a datalogger (model CR5000) equipped with a 1 Gb memory card. Monthly site visits occurred, where data was collected by swapping the full memory card with an empty one. Additionally, real-time data was checked with a lap-top pc to ensure appropriate sensor operation. Fluxes were later calculated offline and corrected using EdiRE (University of Edinburgh, 2003). This approach was used on all data and allowed for the following corrections: despiking and low pass filtering, sonic temperature path correction, sonic flow distortion, rotating velocity signals, sonic temperature density correction, highpass filtering signals, frequency response corrections, sonic temperature correction and density corrections. All corrections were made to the 10-Hz data (time interval of 0.1 second) prior to calculating 30-minute averages. Fluxes were then calculated using the averaged data. More details on the correction procedures can be found on the University of Edinburgh website: http://www.geos.ed.ac.uk/abs/research/micromet/EdiRe.

Task 2: Calculate ET differences prior to and following episodic herbivory by *D. carinulata*

Following the completion of Task 1, the difference in ET between non-herbivory growing conditions, and episodic herbivory growing conditions will be examined. The procedures outlined in Task 1 will generate 30-min corrected averages of ET. These numbers can then be averaged to obtain 60-min ET. The 60-min ET can be summed to obtain daily ET and the daily ET can be summed to obtain yearly ET. In order to quantify herbivory-induced changes in ET, we will calculate the difference between the yearly 2010 ET total and the yearly 2011 and 2012 (both which are highly likely to experience episodic herbivory) ET total. The difference in ET will then be related to potential savings of water (taking into account environmental differences between years, such as environmental demand).

Task 3: Monitor stream flow and daily groundwater oscillations from several locations along the watershed

To determine potential water savings caused by reduced ET rates, the source of water for root water uptake needs to be identified. We propose to determine source water by examining how ET rates correlate to groundwater fluctuations during daytime (active transpiration) and evening (reduced transpiration) periods. The difference in fluctuation rates between these periods should point to the significance of groundwater sources versus vadose zone water. Diurnal groundwater fluctuations will be recorded every minute using a pressure transducer installed in a shallow piezometer (5.08 cm diameter). Data from the transducer will be downloaded to a computer during each site visit. Additionally, stream flow rates will be monitored via USGS real-time online data of gauges located along the lower Virgin River (one near Littlefield, AZ and one near Mesquite, NV). Groundwater and stream flow data will then be compared to ET estimates, determining potential water savings after canopy defoliation.

RESULTS

Following the end of the 2010 growing period, baseline (no beetles presence) ET and rainfall were calculated (Fig. A3.2). Total ET at the Meadowland’s Farm site followed a seasonal bell curve, which is indicative of increasing environmental demand into midsummer, before daytime high temperatures begin to drop in early autumn. The yearly total of 145 cm of ET is the same amount Devitt *et al.*, (1998) calculated at Duck Club during a wet year, approximatley 10 km down river. Before further interpretation of the data can be made, additional analysis of 2010 data needs to be completed. This includes, but is not limited to: 1. analysis of collected soil moisture data, 2. analysis of diurnal groundwater fluctuations at the EC station, 3. analysis of stream flow data collected from the Littlefield, AZ gage, 4. calculation and analysis of potential ET for 2010 and 5. calculation of energy balance from 2010 EC data collection.

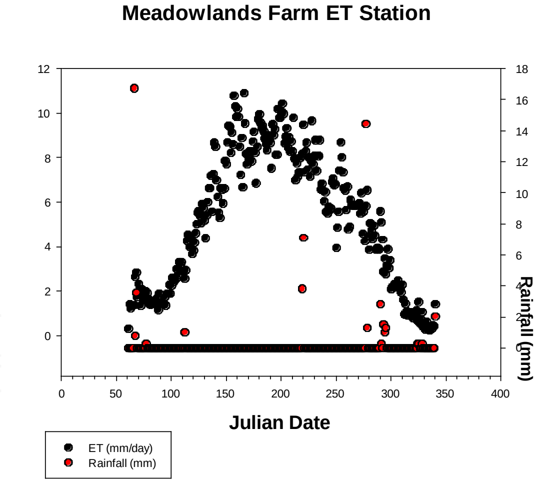


Fig. A3.2. Yearly ET and precipitation for the 2010 growing period.

These analyses, in combination with post-beetle defoliation during the 2011 season, will be crucial in estimating any potential water saving benefits from the use of bio-control to manage tamarisk along the Virgin River riparian corridor.

Finally, these data are intended be used as for comparison to ET measurements being taken by collaborators at locations further down-river at the Mormon Mesa study area. This will allow for a more robust ET data set, which can then be used to scale up estimates with the use of remote sensing techniques.

ON-GOING COLLABORATIVE RESEARCH

During the Spring of 2011, a second ET station was installed in the lower Virgin River Valley, at Mormon Mesa near Overton, NV (36º35’01.86” N 114º19’54.27” W elevation 380 m). The station is operated by Kevin Hultine (Northern Arizona University) and Pamela Nagler (USGS-Tucson). Measurements include stem sap flux of 20 mature tamarisk trees using thermometric heat dissipation sensors (Hultine et al. 2007, 2010) and groundwater fluctuations using a well level recorder installed into a groundwater peizometer. In addition, foliage phenology as it relates to beetle activity, will be monitored with a series of visual and infrared camera systems installed onto a 10 m scaffold tower to be erected near the center of the tamarisk stand during in May of 2011.

Measurements will specifically allow us to quantify alterations in leaf reflectance caused by the tamarisk leaf beetle. We also anticipate installing an Eddy Covariance system, similar to the one described above. In combination, the two stations will improve our spatial coverage of beetle activity and impacts on riparian evapotranspiration in a watershed where bio-control has a relatively high potential to result in water salvage (Hultine and Bush, in press). Likewise, the two systems will allow us to monitor ET in two sub-reaches with potentially large differences in fluvial hydrology.

Water Savings from Tamarisk Biocontrol

Although not addressed specifically in the Statement of Work objectives, the effect of tamarisk transpiration on water resources is of fundamental importance to not only water resource managers in this arid region, but also to wildlife biologists because if tamarisk invasion is lowering groundwater levels, this makes vegetation that is otherwise suitable for wildlife, particularly willow flycatchers, unattractive for nest choice.

We have developed, and continue to develop a baseline dataset for vegetation water use in order to assess water savings anticipated to occur when biological control agents colonize and defoliate large stands of tamarisk, and associated groundwater levels. All data for both ET stations remain preliminary, that is, they are developed only for the period prior to biocontrol agent establishment. Baseline results during 2010, showed approximately 145 cm of ET at the site, with a seasonal rainfall of 5 cm. Give the currently status of *D. carinulata* dispersal and establishment, defoliationof both ET sites will almost certainly occur during the growing period of 2011, just as funding for such work has largely evaporated as a consequence of the inaccurate rading of recent conclusion (Shafroth 2010) that data to-date do not yet support the hypothesis that tamarisk control will lead to water savings in southwestern landscapes. If feasible to continue the Virgin River ET assessment project, this will provide the clearest and most rigorous assessment of such water balance relationships available.

Citations (included in general References section to avoid redundancy)