ASYMMETRIC INTERSPECIFIC COMPETITION BETWEEN SPECIALIST

HERBIVORES THAT FEED ON TAMARISK IN WESTERN COLORADO

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology
(Entomology)

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Logan, Utah

2010

ABSTRACT

Asymmetric Interspecific Competition Between Specialist Herbivores That Feed on

Tamarisk in Western Colorado

by

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Four closely related species of leaf beetles (*Diorhabda* spp.; Coleoptera: Chrysomelidae) have been introduced into the western United States as biocontrol agents for the invasive Eurasian shrub tamarisk (*Tamarix* spp.; Violales: Tamaricaceae). These beetles have since continued to spread and establish throughout the western United States. Another exotic insect, the tamarisk leafhopper (*Opsius stactogalus,* Fieber; Hemiptera: Cicadellidae), had previously become established in these areas and now shares tamarisk as a host plant with the beetles. To assess more carefully the potential for interactions between leafhoppers and beetles, field censuses and cage studies were conducted to determine the phenologies and potential interactions of *O. stactogolus* and *D. carinulata* when attacking *Tamarix ramosissima* (Ledebour) in western Colorado.

The leafhopper underwent development through at least three generations per season, whereas the beetle was shown to develop through two generations per season. Variation in leafhopper abundance was associated with the extent and type of foliar damage to tamarisk trees. Individual trees with greatest *D. carinulata* abundances and subsequent defoliation had significantly reduced *O. stactogalus* abundances thereafter.

Abundance of *O. stactogalus* was also shown to vary significantly among tamarisk plants in cage settings where leafhoppers were given the choice of potted tamarisk plants with ~50% damage to foliage from *D. carinulata* versus undamaged plants. In contrast, *D. carinulata* abundance was not shown to differ strongly in response to *O. stactogalus* damage in the field or in cage experiments. Field results across sites, however, showed similar trends of reduced beetle abundance on plants more heavily attacked by leafhoppers, and larval growth tests suggested slight reductions in larval pupation and adult emergence of *D. carinulata* when grown on *O. stactogalus*-damaged tamarisk. It is not clear if slight tendencies in *D. carinulata* abundance along with much stronger responses in *O. stactogalus* abundance were the result of limited plant material, rather than an induced plant defense. It is clear, however, that these specialist herbivores are interacting in an asymmetric competitive fashion while feeding on the same host plant.

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CHAPTER 1

INTRODUCTION

Tamarisk (*Tamarix* spp.*;* Violales: Tamaricaceae) or saltcedar (as recognized formally by the Weed Science Society of America) is a large deciduous shrub native to Asia, the Mediterranean, and North Africa (Baum 1967). It was originally introduced into the United States in 1823 as an ornamental plant, but eventually became widespread due to its usefulness as a windbreak and for erosion control along railroads and waterways (Harris 1966, Brotherson and Winkel 1986). By 1920 tamarisk had become prevalent throughout the West (Brotherson and Winkel 1986). Today it is known for forming monospecific stands largely devoid of native vegetation, a clear sign that invasion has taken place (Dudley et al. 2000). The cost of the invasion has been estimated to range between $133 and $185 million a year in lost ecosystem services (Brown et al. 1989, Zavaleta 2000). Recent estimates indicate that over 600,000 riparian and wetland hectares are now dominated by Eurasian *Tamarix* species, making the shrub one of the worst invaders in United States history (Gaskin and Schaal 2002).

Tamarisk possesses a combination of attributes that give it a competitive edge over native vegetation. The shrub is highly prolific, producing immense amounts of minute, easily dispersed seeds over extended periods of time (Warren and Turner 1975). Tamarisk is capable of producing seeds by both outcrossing and self-pollinating, and it can also propagate clonally from vegetative growth (Gaskin and Schaal 2002). Other helpful attributes include its ability to tolerate long periods of drought, highly saline soils, heavy flooding, and fire (Lewis et al. 2003). These traits have led to the invasion and

replacement of native vegetation across the western United States. It has been estimated that the shrub has replaced up to 90% of the vegetation in riparian communities along the lower Colorado River and tributaries, once dominated by cottonwood-willow forests (Crins 1989). These altered habitats now support highly reduced plant species diversity (Di Tomaso 1998, Shafroth et al. 2005). Tamarisk also degrades the value of riparian habitat for birds and mammals (Ellis 1995). As a result of tamarisk invading riparian communities in the Southwest, populations of several species of birds and even fish are in decline (Tracy and DeLoach 1999, Dudley et al. 2000, DeLoach et al. 2004)

The prolific nature of the shrub paired with its ability to withstand environmental stress such as drought and fire promote the ability of the plant to establish and spread in newly invaded territory. In addition to demonstrating great resistance and resilience, the shrub is also capable of inflicting stress on competing plant species through high water uptake and the release of excess salt through specialized glands (Kleinkopf and Wallace 1974). Tamarisk can utilize great amounts of saline groundwater by excreting excess salt through specialized glands on the leaves, resulting in increased soil salinity.   The very dense canopy of tamarisk increases the likelihood of fires within stands (Lewis et al. 2003). While vegetative growth of tamarisk aids in re-establishment of the shrub after fire, native species that are less fire tolerant, such as cottonwood and willow, suffer greatly (Lewis et al. 2003).

There are at least 54 extant species of tamarisk; most of these are relatively harmless even when found beyond their native range (Gaskin and Schaal 2002). Only a handful of species have been shown to be capable of invading North America. *T. aphylla* (Linnaeus)*, T. parviflora* (Candolle)*, T. canariensi* (Willdenow)*, and T. gallica*

(Linnaeus) are all considered invasive in western states, including New Mexico, Texas, and California. Two species in particular have been shown to be responsible for the majority of invasion in the United States: *T. ramosissima* (Ledebour) and *T. chinensis* (Loureiro), which are now prevalent in Colorado, Nevada, and Utah (Gaskin and Schaal 2002, Tracy and Robbins 2009). The frequency with which these species occur far exceeds that of the other species combined. Although these particular species are invasive as separate entities, they are most commonly found in hybridized form, and rapid spread of invasive tamarisk can in part be attributed to hybridization (Shafroth et al. 2005). The hybridized genotype of *T. ramosissima* and *T. chinensis* occurs frequently in the United States, but is found in relatively low frequencies in the shrub's native range despite species overlap in regions (Gaskin and Schaal 2002).

Current control tactics for tamarisk include herbicide application, burning, manual removal, and biocontrol implementation. Today there are only a few herbicides available for tamarisk, and when used alone, they have not provided longterm control (Carpenter 2003). Only dicamba (Banvel®), 2,4-D, imazapyr (Arsenal®) or (Habitat®), triclopyr (Garlon®) and tebuthiuron (Spike®) are labeled for the shrub and can provide some measure of control (Grubb et al. 2006, R. Whitesides, personal communication). Herbicides are most effective when combined with other control methods such as mowing, burning, cutting, or root plowing to remove old growth before herbicide application (Grubb et al. 2006). Fire is another method of removal that has proven only partially effective. Fire aids in shorterm control, but tamarisk easily re-sprouts unless other methods of control are applied after a burn (DeLoach et al. 2004). Mechanical removal can be highly effective if most of the taproot is removed (DeLoach et al. 2004).

Mechanical removal has been successful in some state and federal parks such as Island

Acres in Colorado and Dinosaur National Monument in Utah, but it is rarely practical or

cost effective on large plots of land (D. Bean, personal communication).

Biocontrol implementation has proven to be a highly effective method for long-

term control against the spread of tamarisk (Bean et al. 2007). Although complete

eradication of the shrub is highly unlikely, the introduction of small leaf beetles of the

genus *Diorhabda* (Coleoptera: Chrysomelidae) into the United States in 1996 has

resulted in mass defoliation of tamarisk across several western states (Dudley and

DeLoach 2004). The release and establishment of the Mediterranean tamarisk beetle

[*Diorhabda elongata* (Brulle′)], and its sibling species the northern tamarisk beetle [*D.

carinulata* (Desbrochers)], as well as the larger tamarisk beetle [*D. carinata*

(Faldermann)], and the subtropical tamarisk beetle [*D. sublineata* (Lucas)], is currently

underway in an effort to halt the spread of tamarisk in North America (Dudley and

DeLoach 2004, Tracy and Robbins 2009). Collectively these species are known as

tamarisk leaf beetles (Tracy and Robbins 2009). The physiology of each species varies

slightly. In particular, they vary in their seasonal timing of diapause induction, as each

species is adapted to a specific latitude depending on its location of origin (Bean et al.

2007). One species, *D. carinulata,* is currently in use extensively across the U.S. due to

its ability to induce diapause in accordance with the seasonal cues of its introduced range;

these are similar to those of the latitudes found in its native range which includes Turpan

and Fukang China as well as Kazakhstan and Uzbekistan (Tracy and Robbins 2009). The

first open releases of *D. carinulata* in North America took place in 2001, with widespread

defoliation of T. *ramosissima, T. chinensis*, and their hybrid forms occurring in central

Nevada as early as 2002 (Dudley and DeLoach 2004, Dalin and O'Neal 2009).

The establishment of *D. carinulata* at several sites in the western United States, including sites in Nevada, Colorado and Utah, has now led to tens to thousands of acres of defoliated tamarisk (DeLoach et al. 2004). The tamarisk leaf beetle is capable of producing mass defoliation to tamarisk by aggregating in colonies of hundreds to thousands in response to a sex pheromone produced and released by the males (Cosse et al. 2005). Adults of both sexes gather on a chosen host plant to feed and mate, and females produce thousands of eggs (Cosse et al. 2005). The beetle's feeding behavior also makes it capable of mass damage to the host plant (DeLoach et al. 2004). It damages the foliage by scraping tissue off the leaves rather than removing portions of the leaf, a behavior that results in the death of surrounding undamaged foliage (DeLoach et al. 2004). This attribute enables the tamarisk beetle to cause death to more plant tissue than it actually consumes, often resulting in defoliation of the entire shrub and decreased plant growth (Lewis et al. 2003, DeLoach et al. 2004).

An individual female tamarisk beetle can lay up to 400 eggs, but on average each female produces roughly 200 eggs in a lifetime (D. Bean, personal communication, Lewis et al. 2003). The eggs take an average of one week to develop before eclosion and first instars emerge from the egg as tiny black larvae, two to three mm. in length. The larvae will develop through three stadia in approximately 23 days, reaching an approximate length of nine mm as third instars (Lewis et al. 2003). Third instars are easily distinguished by the appearance of a lateral yellow stripe on each side of their body as well as their relatively large size as compared to previous stadia (personal observation). Pupation is completed in approximately seven days resulting in a total

development time from neonate to adult of roughly 30 days (Lewis et al. 2003). Yellow adults of approximately six mm in length emerge with brown lateral stripes on their elytra (Lewis et al. 2003). Adult beetles generally emerge in late April to early May in western states where they have established and drop below the leaf litter for diapause in late August to early September (Bean et al. 2007). The number of generations per season ranges from one to three depending on the species and latitude (Bean et al. 2007).

Feeding by another tamarisk specialist, the tamarisk leafhopper, *Opsius stactogalus* (Fieber)*,* can also reduce tamarisk growth (Liesner 1971, DeLoach et al. 2004). Although the tamarisk leafhopper is substantially smaller than the tamarisk beetle, the leafhopper is capable of damaging the plant by piercing phloem cells and removing their contents from stems using specialized stylets (Wiesenborn 2004). Stem growth was reduced on caged tamarisk plants when fed upon by large numbers of *O. stactogalus* (Liesner 1971, DeLoach et al.b 2004). Chlorosis or discoloration of plant tissue including leaves and stems also results as carbohydrates and other phloem nutrients are extracted by *O. stactogalus* (Wiesenborn 2004). As is the case with many plant and leafhopper species, chlorosis results in yellow or white stippling throughout the plant foliage as the plant reacts to internal cell damage. The characteristic external symptoms are termed hopperburn (Backus et al. 2005). Phloem removal by *O. stactogalus* results in distinctly yellow hopperburn, often accompanied by accumulations of leafhopper-produced honeydew as densities and subsequent feeding damage by the insect increase (Hopkins and Carruth 1954).

The eggs of the tamarisk leafhopper are oviposited under thin layers of bark on tamarisk stems and overwinter in this stage. Eggs typically begin hatching in early May

and the leafhopper develops from neonate to adult through five stadia in approximately one month (Harding 1930). Instars of each stadium are extremely minute and pale in color (personal observation). The initial length of the first instar is approximately 0.08 mm with adults only reaching a length of about 4 mm (Harding 1930). With their slightly larger size and fully developed wings, adults are distinguishable from mature nymphs by their vivid green coloration and dark tipped hemelytra (personal observation). Three to four generations occur per season depending on the location in which they are found (Harding 1930, Leisner 1971, Wiesenborn 2002).

The tamarisk leafhopper and the tamarisk beetle are both host specific to tamarisk, but *O. stactogalus* was introduced unintentionally and its date of arrival into North America is unknown (Harding 1930). Although *O. stactogalus* was first reported in the United States as early as 1907, its introduction likely occurred even earlier, coinciding with the introduction of tamarisk (Harding 1930). This is in contrast to the relatively recent introduction *D. carinulata* and sibling species (Tracy and Robbins 2009). *O. stactogalus* is native to Eurasia but it is common on naturalized tamarisk in many countries, including the United States (Harding 1930).

Although *O. stactogalus* can cause significant damage in a cage setting, the impact of this species in the field for controlling the growth and spread of naturalized tamarisk is generally thought to be insignificant (Liesner 1971, DeLoach et al. 2004, Wiesenborn 2004). In contrast, *D. carinulata* and sibling species have proved to be highly effective at damaging the plant and are even sometimes seen as pests in their native countries when densities increase and result in widespread damage to the plant (DeLoach et al. 2004, D. Bean, personal communication). Even though the role of *O. stactogalus* in controlling

tamarisk is thought to be of little significance, the potential for interaction between the previously established *O. stactogalus* and the recently introduced *D. carinulata* could prove significant in controlling the growth and spread of tamarisk. This could be the case, for example, if the interaction between these specialist herbivores is facilitative and results in greater damage to the plant than herbivory by either species alone.

Facilitation between herbivores can result in greater plant damage if herbivory by one species promotes herbivory by the other. Several studies have found evidence of facilitative interactions in herbivore communities (Strauss 1991, Underwood 1998, Wallen and Raffa 1999, Agrawal 2000); however, competition is more common in herbivore communities (Denno et al. 1995, Denno et al. 2000). For example, a competitive interaction may exist between *D. carinulata* and *O. stactogalus* such that defoliation by the beetle may be significantly lower at sites with high *O. stactogalu*s abundance. Very little research has been performed to test whether the interaction between *D. carinulata* and *O. stactogalus* is facilitative for both species, facilitative for one species but competitive for the other, or competitive for both species.

In this study I have addressed these questions in depth. In Chapter 2, I examine the phenology of both species in the field. Determination of the number of generations per year of each insect species as well as the general seasonal timing of development (i.e. phenology) will provide a better understanding of the potential for these specialists to interact in the field. In Chapter 3, I test the nature of this potential interaction through statistical analysis of field observations combined with cage tests and analyses. These studies were designed to examine whether evidence exists either for or against a facilitative or competitive interaction between these two species in their introduced range,

and to provide improved understanding of the nature of any such interaction and its effect on tamarisk. My ultimate goal was to improve our current understanding of the combined impact that these insect species have in controlling the spread of this highly invasive plant species (Gaskin and Schaal 2002).

I hypothesized at the outset of this study that interactions between the two species do indeed exist. I further hypothesized that these interactions are a combination of both facilitative and competitive effects, resulting in an overall asymmetric interspecific interaction. Thus I predicted that prior damage from *O. stactogalus* deters feeding by *D. carinulata,* resulting in lower beetle defoliation rates on tamarisk trees with high *O. stactogalus* abundance and damage (i.e., there is a competitive effect of the leafhopper on the beetle). I also predicted that defoliation by *D. carinulata* will increase host attractiveness for *O. stactogalus*, and consequently greater *O. stactogalus* abundances will be found on tamarisk trees with previous damage by *D. carinulata* (i.e., there is a facilitative effect of the beetle on the leafhopper). Thus I expected to see less damage to tamarisk at sites with high abundances of *O. stactogalus* as opposed to *D. carinulata*. In the next two chapters, I first present the phenologies of the two insect species and then test these initial hypotheses through field observations and caged host choice testing performed over the summers of 2008 and 2009 at sites in western Colorado.

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CHAPTER 2

SEASONAL PATTERNS OF ABUNDANCE OF SPECIALIST HERBIVORES ON TAMARISK IN WESTERN COLORADO

Abstract

Seasonal patterns in the abundance of two species of specialist herbivores that feed on the invasive shrub tamarisk, *Tamarix ramosissima* (Ledebour), were determined over two summers (2008 and 2009) at five sites along tributaries of the Colorado River in western Colorado. The tamarisk leaf beetle, *Diorhabda carinulata* (Desbrochers), and the tamarisk leafhopper, *Opsius stactogalus* (Fieber), are both known to specialize on tamarisk as a food source. These species co-exist on this shared resource in their native habitat, but the interaction of the two in their introduced range has not been well studied. Determination of their phenology, including including timing of peak abundance and spatial distribution over two seasons provides information on their interactions in one area of their introduced range. This study provides evidence that *O. stactogalus* undergoes development through three generations per season as found previously by Harding (1930) and Wiesenborn (2002). Peak nymphal abundance occurred in late June to early July resulting in peak adult abundances in mid to late July during both seasons. *D. carinulata* was shown to develop through just two generations per season. Peak larval abundance occurred in early to late June resulting in peak adult abundances in mid to late July during both years. These findings provide evidence that these species overlap frequently through space and time in their introduced range while feeding on their shared host plant, tamarisk. Such overlap may result in an interaction between these specialist herbivores as mediated by their host plant.

Introduction

Four closely related species of leaf beetles (*Diorhabda* spp.; Coleoptera: Chrysomelidae) have been introduced into the western United States as biocontrol agents for the invasive Eurasian shrub tamarisk (*Tamarix* spp.; Violales: Tamaricaceae) (Tracy and Robbins 2009). The first open releases of the beetles took place in 2001 using the species *Diorhabda carinulata* (Desbrochers). This beetle has since continued to spread and establish throughout the western United States (Dalin and O'Neal 2009). Previously another insect, the tamarisk leafhopper (*Opsius stactogalus,* Fieber; Hemiptera: Cicadellidae), had become established accidentally in these areas and now shares tamarisk as a host plant with the beetles (Wiesenborn 2002). To assess more carefully the potential for interactions between leafhoppers and beetles, here I present the results of field censuses to determine the phenologies of *O. stactogolus* and *D. carinulata* attacking *Tamarix ramosissima* (Ledebour) in western Colorado where the beetle has been introduced in recent years.

The four *Diorhabda* species vary in their seasonal timing of diapause induction, as each species is especially adapted for a specific latitude in its native range (Bean et al. 2007). Variations in diapause characteristics lead to variable phenologies of the beetles, with some populations having a single generation each year and others having up to three generations per year. The physiology and phenology of the beetles may vary not only among species; intraspecific differences also may occur among populations established in different areas of western North America, associated with varying abiotic and biotic factors among these areas. The recent establishment of *D. carinulata* in western Colorado provides the opportunity to examine the phenology of this particular species in this part

of western North America. The leafhopper is well established in western Colorado, thereby allowing for a comparison of its phenology (previously found elsewhere in western North America to include several generations per year; Harding 1930) with the phenology of the beetle.

Although the introduction and subsequent establishment of *O. stactogalus* in the United States was accidental, this phloem feeding leafhopper is now abundant along the Colorado River and tributaries (Wiesenborn 2002). Both *O. stactogalus* and *D. carinulata* are specialist herbivores associated with tamarisk, but in contrast to *D. carinulata*, *O. stactogalus* is not known to be effective at reducing either tamarisk growth or stand density (Liesner 1971, DeLoach et al. 2004). However, the leafhopper's ability to reduce the growth of this highly invasive plant species may increase or decrease now that it shares tamarisk in North America with *D. carinulata*. Similarly, the biocontrol potential of the beetle may be influenced by the presence of the leafhopper. A first step in considering possible competitive or facilitative interactions between these two herbivores of tamarisk is to establish the timing of their life cycles in exploiting the host plant under natural conditions where they occur together in their introduced range. The field censuses of tamarisk stands in western Colorado that are presented here address this first step.

Materials and Methods

Field censuses of individually marked tamarisk trees were conducted at study sites along Colorado River tributaries in western Colorado in 2008 and 2009. The censuses (described more fully below) were conducted at five sites over the two summers to determine seasonal patterns of nymphal or larval and adult abundance of *O. stactogalus* and *D. carinulata*. The censuses were conduced approximately every two

weeks beginning in late May of 2008 and mid May of 2009. The interval between censuses was shortened to once weekly in July of both years because the abundance of *D. carinulata* increased rapidly during this month. Censuses ended in late July 2008, and in early August 2009. During each census, 25 marked trees per site were measured for insect abundance.

Study sites

The five sites were chosen based on accessibility, recommendation by BLM and private landowners, and tamarisk stand density and size. The locations of these sites encompass a range of approximately 160 kilometers from the northernmost site (Stan Young Un-burned) to the southernmost site (Bedrock).

The three northern sites were located approximately 32 kilometers west of Grand Junction, Colorado, on Salt Creek, a small tributary of the Colorado River. The Salt Creek site (39°13' N, 108°53' W), referred to hereafter as Saltcreek, was approximately 8 kilometers north of two sites referred to as the Stan Young sites. These sites occurred on private land, where approximately 12 hectares of tamarisk underwent a controlled burn in 2007 (Stan Young Burned site, 39°17' N, 108°51' W) and another large stand of approximately 16 hectares at a distance of approximately 2 kilometers north of the burned stand remained unburned (and is hereafter referred to as Stan Young Unburned, 39°18' N, 108°51' W).

The two southern sites, Gateway (38°34' N, 108°55' W) and Bedrock (38°17' N, 108°53' W) were approximately 80 kilometers and 129 kilometers south of Grand Junction, respectively. The Gateway site was located on the Dolores River approximately 8 kilometers east of the town of Gateway, Colorado. The Bedrock site was 48 kilometers

south of the Gateway site and 4 kilometers south of the town of Bedrock, Colorado. The Bedrock site was located on the San Miguel River, a tributary of the Dolores River, which joins the Colorado River in southern Utah.

Geomorphology differed among sites. The Bedrock, Gateway, and Saltcreek sites occurred in canyons of varying dimensions whereas the Stan Young sites were located in an open valley. All five sites were located on flat rocky riverbanks with dense tamarisk stands (plant spacing consisted of 0.5 to 2 meters between canopies). Tamarisk trees ranged in size from 0.5 to 8 meters in both width and height. Average tree size was 2.5 meters in width and 3 meters in height. Soil type and ground cover varied slightly among sites. Gateway and Bedrock soils were sandy while Saltcreek and Stan Young sites had denser soils with more clay. Although most of the vegetation present was tamarisk, other desert shrubs and herbaceous species also grew at each site. These included big sage *Artemisia tridentata* (Nutt), greasewood *Sarcobatus vermiculatus* (Nees), grey rabbitbrush *Chrysothamnus nauseosus* (Pallus), lambsquarters *Chenopodium album* (Krasan) and various non-native invasive species such as Russian knapweed *Acroptilon repens* (L.), Russian thistle *Salsola iberica* (Sennen and Pau), kochia *Kochia scoparia* (Roth), and downy brome *Bromus tectorum* (Linnaeus). Riparian species such as Gooding's willow *Salix gooddingii* (Ball) and Fremont cottonwood *Populus fremontii* (Watson) were infrequent at the sites.

At each of the five sites, 25 tamarisk trees were marked individually to be censused repeatedly throughout the summers of 2008 and 2009. Marked tree size was not controlled for in this study. Trees were randomly chosen at 10 meter intervals along transects in various directions at distances up 200 meters from a centrally located release

tree at which *D. carinulata* beetles (~5,000 per site) had been introduced to the site in a previous year. These releases were made in 2006 at the Gateway and Saltcreek sites and in 2007 at the Bedrock and the Stan Young sites. By 2008, beetle populations were established at all sites. Population numbers were low early in the summer of 2008 at Salt Creek and Stan Young sites. At Bedrock and Gateway, high numbers of migrating beetles had arrived in late summer of 2007 (personal observation). These beetles had likely migrated up the Dolores River from a much larger population originally established in 2004 southwest of the Gateway and Bedrock sites, on the confluence of the Dolores and Colorado River in southeast Utah near Moab.

Censuses to estimate insect abundance

Leafhopper abundance was estimated with branch tap counts of four randomly chosen branches per tree. A single branch in each quarter section of a tree (for a total of four branches per tree) was chosen randomly (i.e., blindly, without consideration for the extent of either beetle or leafhopper damage present). Branches varied randomly in size, with the exception that unusually large branches were excluded because they could not be sampled well by the tap count method. All branches were chosen from an approximate height of 1.5 to 2 meters on the tree. Leafhopper counts were taken after each branch was gently shaken over an upright rectangular plastic container (15 by 15 by 30 centimeters) by tapping the branch several times on the rim of the container. The bottom of the container was fitted with a white cloth panel to increase the visibility of the very small leafhoppers once they fell from the branch into the container. After a branch had been tapped three times against the container rim, the *O. stactogalus* that had fallen to the bottom of the container were counted as nymphs or adults. For each tree sampled, a

single estimate of leafhopper abundance was obtained for adults, and for nymphs, by adding together the counts for the four branches sampled. These estimates (N = 25) were used to calculate the mean abundance of leafhoppers (per four branches) at each census site.

Beetle abundance was estimated using in situ estimates of total adult and larval abundance over the entire tree canopy. Three observers each carefully scanned each tree by walking around the tree and examining individual branches to estimate the total abundance of adults and larvae. A consensus of an approximate beetle density estimate for each tree was then reached among the three observers both for adults and for larvae, and was recorded as 0, 1, 10, 100, 500, 1000, or 1500 individuals (determined by selecting one of the seven levels of abundance that most closely matched the consensus estimate). An estimate of mean abundance for a site at each census date was then determined from the 25 estimates (for individual trees).

Results and Discussion

Leafhopper phenology

The phenology of *O. stactogalus* was first documented by Harding (1930), who studied this species near Lawrence, Kansas, and found it to have three generations per year. Harding (1930) observed nymphs to develop from egg to adult over the course of approximately one month under field conditions, while passing through five nymphal stadia. Wiesenborn (2002) confirmed that three generations per year are completed also in southern Nevada. Liesner (1971), however, reported four generations per year in New Mexico.

Female *O. stactogalus* insert their eggs into tamarisk stems, just under the surface

of the bark (Harding 1930). The egg is the overwintering stage and eggs begin hatching as timed with the availability of green plant material in mid to late spring (Wiesenborn 2002). Harding (1930) first observed nymphs on 9 May 1929 in Kansas. Although nymphs were not observed in censuses conducted in May 2008, this result was likely due to sampling error (i.e. unfamiliarity with the very small size of early instar nymphs in samples resulted in missed observations) on the first monitoring date of the first season in the study (Fig. 2.1). Nymphs were observed on tamarisk trees in censuses taken in mid-May 2009 in western Colorado (Fig. 2.1).

The emergence of *O. stactogalus* nymphs from overwintering eggs closely matches the resumption of growth (budburst and leaf expansion) by tamarisk in the spring, as *O. stactogalus* feeds on phloem (Harding 1930, Wiesenborn 2004). New, young leaves typically first appear on tamarisk in late April in western Colorado (*personal observations*). Overwintering *O. stactogalus* eggs likely begin hatching at or soon after this time. The very low numbers of nymphs that were found during censuses in mid-May 2009 thus represented the first (overwintering) generation. Consistent with observations by Harding (1930) in Kansas, and consistent also with his estimate of approximately one month's time to mature from egg to adult, the earliest of these nymphs to hatch had reached adulthood by early June 2009, when adults were first detected in censuses (Fig. 2.2). Considerably more first generation adults were present on trees by mid-June (Fig. 2.2). First generation adults were also present in considerable numbers in 2008 when censusing was undertaken in mid-June (Fig. 2.1). The large numbers of nymphs that were subsequently found on the trees in late June (Fig. 2.1) likely represent a second generation produced by first generation adults. This second generation of nymphs had

largely disappeared from the trees by mid-July 2008, during which time adult numbers on the trees swelled as the nymphs matured (Fig. 2.1). As adult numbers moderately declined in late July, high nymphal abundance on the trees was again recorded and presumably represented a third generation (Fig 2.1). Based on the observations of Harding (1930), these third generation nymphs likely reached adulthood in late August through September (censusing, however, ceased in late July). The third generation of adults was likely the generation to produce overwintering eggs that hatched the following spring (i.e., in 2009).

Seasonal patterns for the leafhopper in 2009 were largely consistently with those for 2008, although leafhopper densities on the trees were considerably lower on average throughout the second summer (2009). Thus, as in 2008, peak numbers of nymphs, representing second and third generations, were recorded in mid June and late July-early August (Fig. 2.2). Also as in 2008, numbers of adults on the trees overall increased over the summer, with peak numbers occurring in the later half of July and early August during the final rounds of censuses in 2009 (Fig. 2.2).

Further study is needed during late summer and fall in western Colorado to test for the possibility of a fourth generation (as observed farther south in New Mexico by Leisner 1971). The existence of three generations in western Colorado with timing as documented in the present study, however, is consistent with previous studies of the phenology of this leafhopper by Harding (1930) and Wiesenborn (2002).

In contrast to seasonal patterns of abundance of nymphs in 2008 and 2009, seasonal patterns of adults of *O. stactogalus* were more characterized by a general increase in abundance over the summer rather than by a clear early season peak preceding a later,

second mid-summer peak (Figs. 2.1 and 2.2). These patterns for adults likely reflect considerable overlap in the occurrence of successive generations of adults over the summer, as noted previously by Harding (1930) who estimated that adults may live up to a month in the field. Adult longevity combined with the completion of at least three generations of *O. stactogalus* adults per season in western Colorado leads to the expectation that generations will overlap extensively in time, in fashion similar to other multivoltine leafhopper species (Duan and Messing 2000).

Leafhopper abundances varied considerably among the five sites in 2008. Numbers of nymphs were especially high throughout the season at the Stan Young unburned site, and particularly low late in July at the Bedrock site (Fig. 2.2). Curiously, adults (but not nymphs) were abundant at the Bedrock site in late July, although they were considerably less abundant than at the Stan Young unburned site (2008). Despite variations in abundance among sites, seasonal patterns were fairly similar among the sites in 2008 (Fig. 2.1). In contrast, seasonal patterns were less similar among sites in 2009 (Fig. 2.2). The difference between years likely reflects the very low abundance of *O. stactogalus* across all sites in 2009, which hindered the ability to detect strong seasonal changes in abundance at any given site. In general, northern sites (i.e., Saltcreek, Stan Young Burned and Un-burned) tended to have greater abundances of both nymphs and adults in both years in comparison with the two southern sites (Gateway and Bedrock) (Figs. 2.1 and 2.2). The cause of the significant decrease from 2008 to 2009 in *O. stactogalus* population density is not known. Although several parasitoids attack *O. stactogalus*, mortality by parasitoids has not been shown to be a strong determinant of population density (Wiesenborn 2004). All sites experienced significant decreases in leafhopper

abundance from 2008 to 2009, perhaps reflecting differing weather conditions between years. Although damage by *D. carinulata* may have had influence on decreasing leafhopper abundances, it seems unlikely to be the major cause due to the wide range of beetle damage among sites in 2008 (i.e. sites with very little damage by the beetle experienced the same lower abundances of the leafhopper as sites with higher damage). Further investigation is necessary, however, to determine the cause(s) of this broad population decline throughout the study area.

Beetle phenology

The establishment and phenology of *D. carinulata* and three sibling species has been documented at several locations across the western United States as part of the effort over the past ten years to introduce these insects as biocontrol agents of tamarisk (Tracy and Robins 2009). Not all introductions of these species have led to successful establishment and subsequent control of tamarisk, but establishment has occurred at a number of sites, including the sites in western Colorado studied here. Each of the four species is from Eurasia, but they differ in the latitudes at which they occur in their native ranges across Asia and Europe. Therefore these four species of *Diorhabda* have varying temperature and daylength requirements associated with diapause and adult emergence in the spring (Lewis et al. 2003, Bean et al. 2007, Tracy and Robins 2009). *D. carinulata* is native to parts of China and Kazakhastan, at similar latitudes to those of areas of introduction in Nevada, Utah, and Colorado where *D. carinulata* has successfully established (Tracy and Robins 2009).

D. carinulata overwinters as an adult. Emergence in the spring must closely match the budding of green plant material on tamarisk, as is true for egg hatch of *O. stactogalus*

(Lewis et al. 2003, Bean et al. 2007). Such timing of adult emergence may be linked to increasing temperature and daylength in the spring (Bean et al. 2007). Larval development from a neonate to adult is approximately 25 days and includes three instars (Lewis et al. 2003). One or two generations of *D. carinulata* occur per year, depending on timing of adult emergence in relation to photoperiod (i.e., daylength) (Bean et al. 2007). For example, if adults emerge in summer before daylength shortens below approximately 14 h 39 m, they will become reproductively active and produce a second generation in the same summer (Bean et al. 2007). Adults that emerge after daylength has dropped below this critical length will enter reproductive diapause. Reproductively active adults have been estimated to live for 12 to 18 days, whereas adults that enter reproductive diapause may live up seven months (Lewis et al. 2003, Bean et al. 2007).

Low numbers of overwintered adults were found on trees during censuses in late May 2008 in the present study (Fig. 2.4). Numbers of adults remained low at all sites throughout June. These adults produced a first generation of larvae that peaked in abundance on the trees in late June (Fig. 2.3). The highest densities of larvae occurred at the Gateway site, with moderate densities at Bedrock, and low densities at the three northern sites (Fig. 2.3). These two southwestern sites also had the highest numbers of adults during June 2008. These sites were closest to the Utah border and likely had experienced a large influx of adults dispersing along the Dolores River during the previous summer of 2007 (personal observation). Numbers of larvae on the trees in 2008 declined overall during July, as individuals pupated and emerged as first generation adults that peaked in numbers in late July (Fig. 2.3). Because census were discontinued in late July, it was not possible to confirm fully the occurrence of a second generation of

larvae in 2008. The increasing numbers of larvae in late July at the Bedrock site, however, are suggestive of a second generation (Fig. 2.3).

Census data for 2009 are clearer in indicating that a second generation of beetle larvae occurred at the two Stan Young sites (Fig. 2.4). Overwintered adults were numerous on trees at the Gateway and Bedrock sites during May 2009 following higher densities in 2008 at these two sites (Fig. 2.4). By mid-June adult numbers had dropped to low levels at all sites, while first generation larvae were abundant on the census trees (especially at Gateway site; Fig. 2.4). Thus the first generation larvae of the season emerged slightly earlier in 2009 than in 2008. These larvae developed under long daylength conditions and were cued to emerge as fully reproductive adults in early to mid July 2009 (Fig 2.4). The eggs that these adults produced probably began hatching in late July, leading to a second generation of larvae that was especially large and was peaking in abundance at the Stan Young Unburned site in early August when censuses were discontinued (Fig. 2.4)

Daylength considerations suggest that the second generation adults that developed in August from these larvae emerged in reproductive diapause. Even though previously emerged *D. carinulata* adults may temporarily remain reproductively active as daylength drops below a minimum threshold, adults that emerge thereafter from pupation (i.e., after daylength has shortened below this minimum threshold) will emerge in diapause (Bean et al. 2007). Reproductively active adults from the first generation become diapausal after a lag time of 14 days following the drop of daylength below the critical threshhold of 14 h 39 m (Bean et al. 2007). Daylength shortened below this critical threshold on 17 July 2008 and 18 July 2009 (U.S. Naval Observatory 2010), and most second generation

adults emerged more than 14 days later. Therefore the development of the second generation of *D. carinulata* in western Colorado resulted in a generation of adults cued for diapause at the time of emergence from pupation (Bean et al. 2007).

Many of the late emerging first generation adults at the study sites were also likely cued for diapause at the time of emergence. Earlier emerging first generation adults likely stopped reproducing a short time (~14 days) after they entered into shortened daylength conditions (Bean et al. 2007). Daylength considerations thus suggest that relatively few second generation adults are produced in western Colorado, and therefore most of the overwintering population of adults are first generation adults of the previous summer. In addition, first generation adults would have more time during the summer, and thus a far better chance, of building adequate fat bodies before the onset of winter than would second generation adults. Hence the more abundant first generation adults can be expected to survive the winter at higher rates than the less abundant second generation adults.

D. carinulata has also been confirmed to produce two generations per season at sites in California, Nevada, Wyoming, Utah and southeastern Colorado. This species is multivoltine in its native range, sometimes even producing three generations per season such as in Fukang, China (Lewis et al. 2003). In contrast this species has been found to be univoltine in southern regions of North America such as Texas, where establishment failed as the likely result of critical daylength never exceeding 14 h 39 m required for inducing reproductive behavior (Lewis et al. 2003, Bean et al. 2007).

Once adults are fully cued for diapause, they will drop from the tamarisk on which they have fed and submerge below the leaf litter under their host plant until the following

April or May when increasing daylength and temperature signal availability of host plant tissue once more (Lewis et al. 2003). The precise timing of submergence or emergence from the leaf litter by diapausal beetles cannot be determined for the sites in western Colorado where this study took place because censuses were not conducted before May or after early August, The successful overwintering of this species at these sites, however, indicates that *D. carinulata* responded properly through diapause to the length and severity of winter and summer conditions in western Colorado.

Conclusions

The broad overlap in the phenologies of *D. carinulata* and *O. stactogalus* that occurs in western Colorado provides much potential for possible positive or negative interactions between these two species as they share a common food source, tamarisk. Both species are present throughout the season, and each can achieve high numbers on the tamarisk trees at individual sites. In particular, herbivory from high numbers of first generation leafhoppers early in the season may influence subsequent herbivory by *D. carinulata* as the overwintered beetle adults produce a first generation of larvae that attack the trees in late June and early July. Similarly, the damage inflicted by the beetles in late June and early July may influence the amount of herbivory that tamarisk sustains from the second generation of leafhoppers later in the summer. Additional analyses and studies are needed to test for whether such interactions (either positive or negative) may occur between the leafhopper and beetle as they share tamarisk as a host plant.

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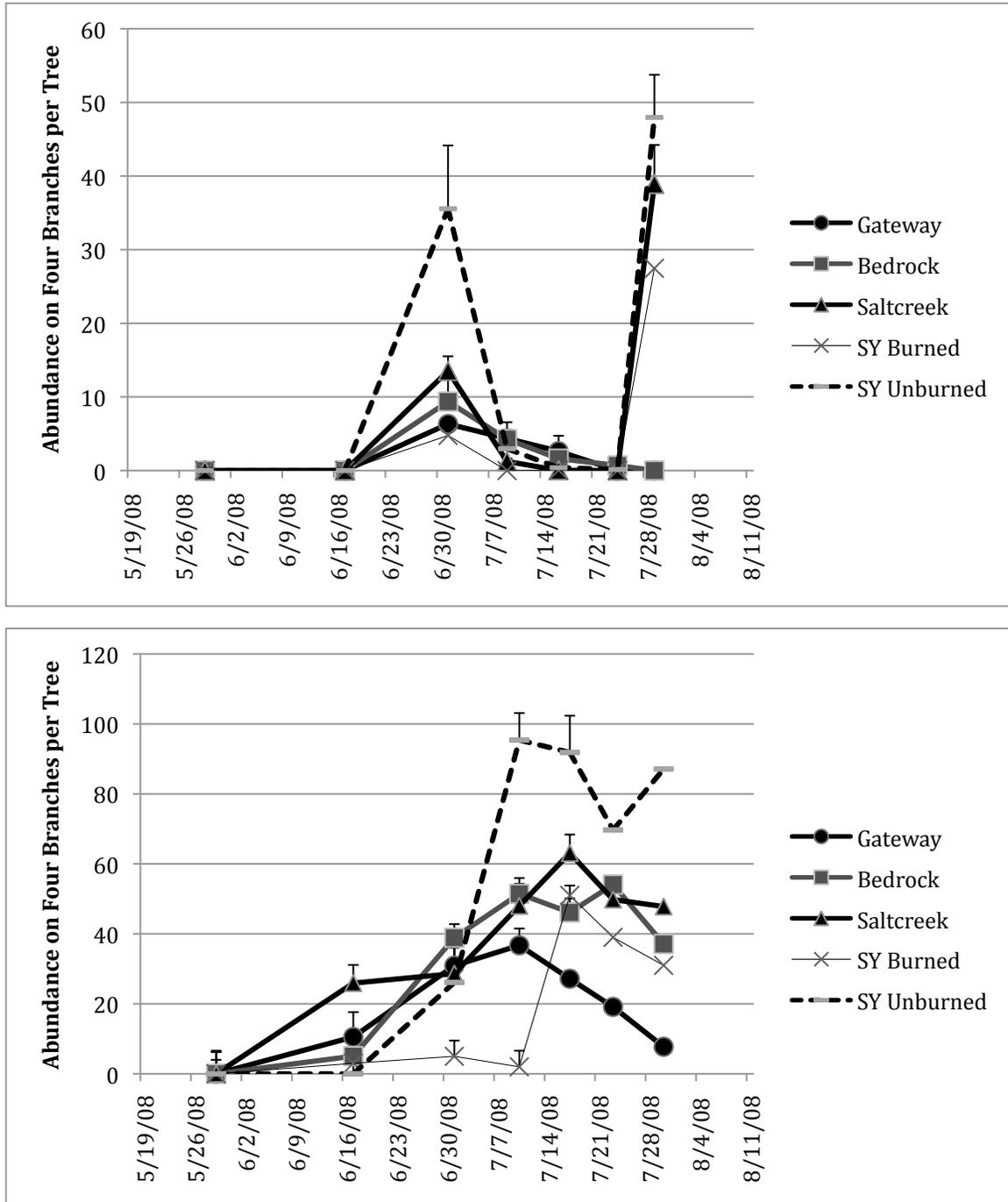
Fig. 2.1. Mean abundance of *O. stactogalus* nymphs (top) and adults (bottom) across sites, June-July 2008. See text for details on site locations and sampling methods. Error bars are shown for one standard error (in some cases standard errors are too small to appear in the figure).
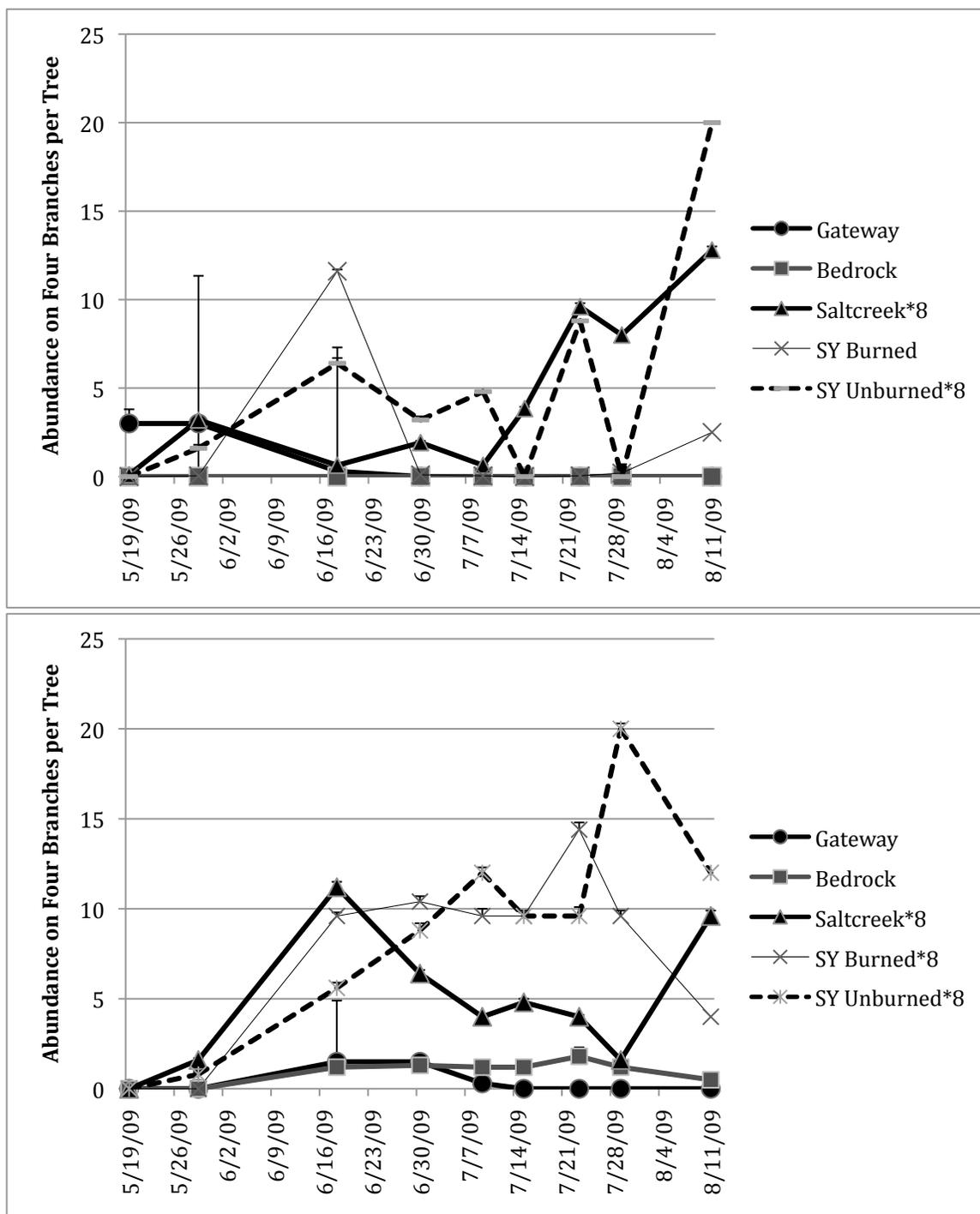
Fig. 2.2. Mean abundance of *O. stactogalus* nymphs (top) and adults (bottom) across sites, May-Aug. 2009**.** See text for details on site locations and sampling methods. Error bars are shown for one standard error (in some cases standard errors are too small to appear in the figure).
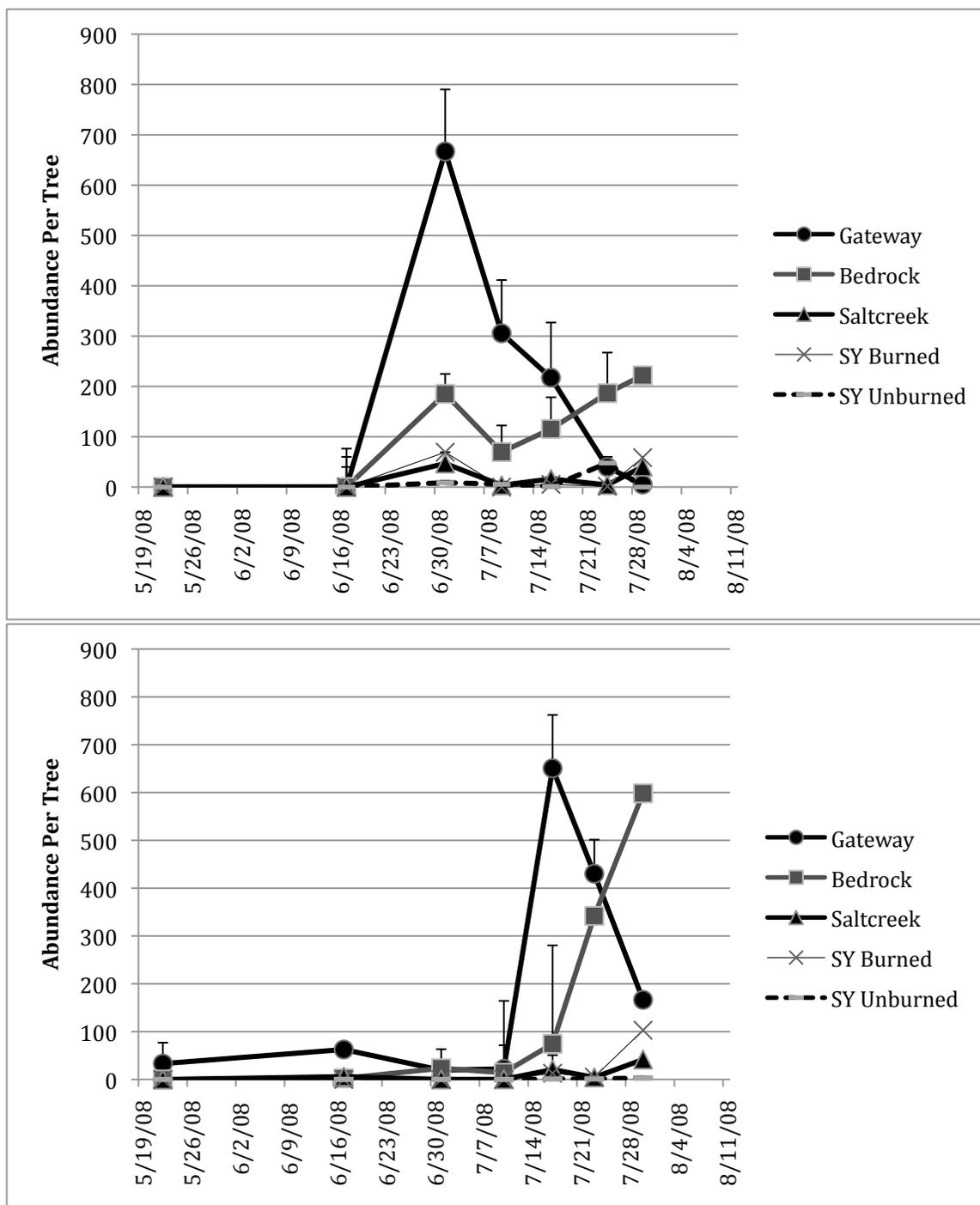
Fig. 2.3. Mean abundance of *D. carinulata* larvae (top) and adults (bottom) across sites, June-July 2008. See text for details on site locations and sampling methods. Error bars are shown for one standard error (in some cases standard errors are too small to appear in the figure).
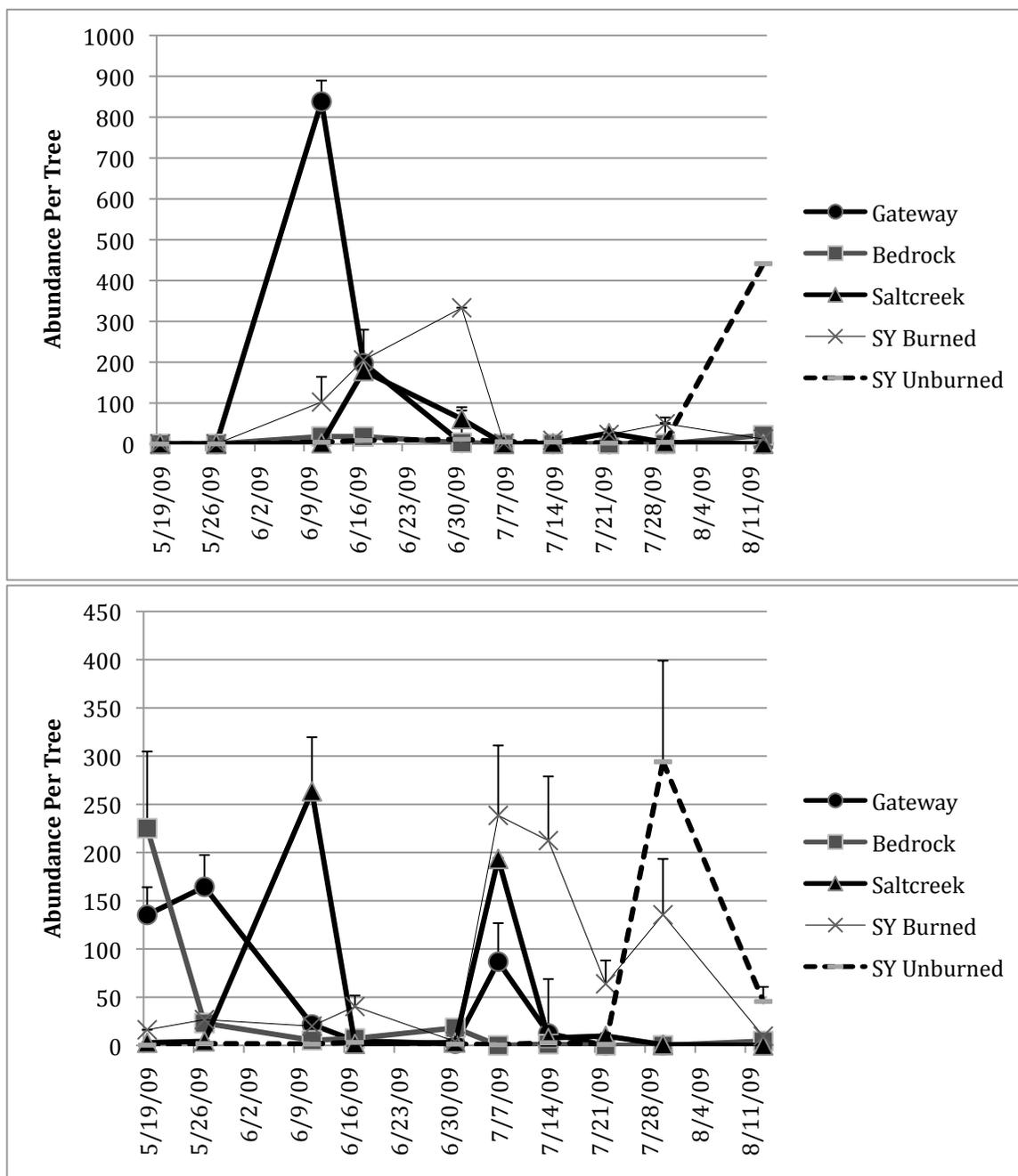
Fig. 2.4. Mean abundance of *D. carinulata* larvae (top) and adults (bottom) across sites, May-Aug. 2009. See text for details on site locations and sampling methods. Error bars are shown for one standard error (in some cases standard errors are too small to appear in the figure).

CHAPTER 3

ASYMMETRIC INTERSPECIFIC COMPETITION BETWEEN SPECIALIST HERBIVORES THAT FEED ON TAMARISK IN WESTERN COLORADO

Abstract

The seasonal patterns and potential interactions of *Diorhabda carinulata* (Debrochers)*,* the tamarisk beetle*,* and *Opsius stactogalus* (Fieber), the tamarisk leafhopper, were examined on *Tamarix ramosissima* (Ledebour), tamarisk, over two summers in 2008 and 2009, at five sites along tributaries of the Colorado River in western Colorado. Field observations were combined with cage experiments to test if these specialist herbivores are interacting and to determine whether the interaction is competitive, facilitative, or neutral as mediated by feeding on the same host plant. Variation in species abundance associated with and in response to the percent and type of foliar damage at field sites was measured to test for possible interactions. Individual trees showing the greatest *D. carinulata* abundances and subsequent defoliation had significantly reduced *O. stactogalus* abundances thereafter. *O. stactogalus* abundance was also shown to vary significantly among tamarisk plants in a cage setting where leafhoppers where given the choice of ~50% *D. carinulata* damaged potted tamarisk plants versus non-damaged plants. In contrast, *D. carinulata* abundance was not shown to differ strongly in response to *O. stactogalus* damage in the field or in cage experiments. However, field results across sites showed similar trends of reduced beetle abundance on plants more heavily attacked by leafhoppers, and larval growth tests demonstrated slight reductions in larval pupation and adult emergence of *D. carinulata* when grown on *O. stactogalus* damaged tamarisk. It is not clear if slight tendencies in *D. carinulata* abundance along with much stronger responses in *O. stactogalus* abundance were the

result of limited plant material, rather than an induced plant defense. It is clear, however, that these specialist herbivores interact in an asymmetric competitive fashion while feeding on the same host plant.

Introduction

Plants are attacked by a variety of herbivorous insect species, and interactions among coexisting herbivores sharing a host plant are well documented (Price 1997). Understanding such interactions is important in the practice of biological control of weeds, in which more than one species of insect is often introduced to attack a weed (Denoth et al. 2002, McFadyen 1998). Here I examine the nature of interactions between populations of two insect species that attack tamarisk, *Tamarix ramisissima* Ledebour (Violales;Tamaricaceae), a weedy, introduced tree that now infests vast areas of riparian habitat in western North America (Gaskin and Schaal 2002, Dudley and DeLoach 2004, Tracy and Robbins 2009). One herbivore is the leaf beetle, *Diorhabda carinulata* Desbrochers (Coleoptera: Chrysomelidae), introduced from Asia and recently widely released throughout western North America for biological control of tamarisk (DeLoach et al. 2004, Tracy and Robbins 2009). The second herbivore is the leafhopper, *Opsius stactogalus* Fieber (Hemiptera: Cicadellidae). It is also non-native and attacks tamarisk in its endemic range (Harding 1930). The leafhopper has become broadly distributed throughout western North America after accidental introduction many decades ago (Harding 1930, Wiesenborn 2004), and co-occurs at many locations with the introduced beetle.

The tamarisk leaf beetle and the tamarisk leafhopper may interact negatively through competition, or may interact positively through facilitation, in sharing the host

plant or interactions may be neutral. Competition for shared resources occurs often across insect taxa when plant material is limited or the plant responds with an induced defense to herbivory (Denno et al. 1995, Karban and Baldwin 1997). Consequently, the previous feeding damage from one herbivore species sustained by a host plant may negatively affect its subsequent use by a second herbivore species. Many herbivores elicit biochemical, physiological, or morphological changes in their host plant (Kaplan and Denno 2007). These responses may result in resistance to subsequent attack by co-occurring herbivores (Viswanathan et al. 2005, Kaplan and Denno 2007). Competitive relationships between herbivores can be reflected by lower numbers of one herbivore species attacking a host plant when that plant has previously been attacked by another herbivore species. Such interactions may be asymmetric in that one species has greater effect on its competitor than does the other (Price 1997). Tamarisk has not been documented to display induced defenses to insect damage. Some work has been done previously to compare the rate of growth and survival of *D. carinulata* larvae when grown on tamarisk previously damaged by this beetle versus on undamaged tamarisk, but these results were non-significant and not published (T. Dudley, personal communication).

In other cases, plant responses to attack from one herbivore species may make the plant more susceptible to attack by co-occurring herbivores; i.e., a facilitative relationship may occur between herbivore species sharing the host plant. Few studies have provided evidence of facilitation among co-occurring herbivores (Denno et al. 1995). However, there is some evidence that damaged plants may be more susceptible to subsequent attack following the release of plant volatiles (Barbosa and Schultz 1987, Damman 1989, Pallini

et al. 1997). Elevated abundances of an herbivore species on host plants previously damaged by another herbivore species (versus on undamaged plants) may reflect a facilitative interaction. In the absence of strong interactions (either competitive or facilitative) between co-existing herbivore species, the abundances of each species on a particular host plant may bear little relation to how heavily that host plant may have been attacked previously by the other herbivore species.

In this study, I examine through both field observations and cage experiments whether the tamarisk beetle and tamarisk leafhopper respond in their intensity of attack to individual host plants previously injured by the other herbivore. Understanding the nature of the interactions between these specialist herbivores is important for understanding their combined potential for biological control of tamarisk across western North America. Although the beetle has already proven quite successful as a biocontrol agent against this highly invasive plant species, the role that the leafhopper plays either in support of the beetle or in undermining the impact of the beetle is less clear (Bean et al. 2007, Tracy and Robbins 2009). Close examination of the effect of colonization of host plant individuals by one insect on the future colonization of the other, as mediated through feeding damage, is necessary to evaluate the relationship between these specialist herbivores.

Materials and Methods

Field observations

Tests of the beetle's and leafhopper's responses to each other's feeding damage were drawn from field censuses of individually marked tamarisk trees conducted at five study sites along Colorado River tributaries in 2008 and 2009 (see Chapter 2 for full description of these sites). The sites were chosen based on accessibility, recommendation

by BLM, and private landowners, as well as tamarisk stand density and plant size. They were spread over a range of approximately 160 kilometers, and included three northern sites (Saltcreek and Stan Young Unburned and Burned [with a controlled fire in 2007]) and two southern sites (Gateway and Bedrock). Each site was located on a flat rocky riverbank with dense tamarisk populations (0.5 to 2 meters between plant canopies). Tamarisk trees ranged in both width and height from 0.5 to 8 meters. Average tree size was 2.5 meters in width and 3 meters in height.

Censuses were conducted to estimate insect (beetle and leafhopper) abundance and damage. The beetle overwinters as an adult and reproduces in early summer. A first summer generation of adults is produced in July following completion of larval development, and a second generation in August (late maturing, first generation adults, however, enter diapause without reproducing; see Chapter 2 for further explanation). The leafhopper overwinters as an egg and completes nymphal development for a first generation in May-June, completes a second generation in late July, and a third generation in late August-September (see Chapter 2 for further explanation). Although beetles had been introduced previously to the study sites, few overwintered adults occurred at any of the sites during June 2008, and most early summer foliar damage to tamarisk was caused by leafhoppers. This scenario allowed for a test of beetle response in late June and July 2008 to previous leafhopper feeding damage to marked tamarisk trees at the study sites. Similarly, heavy previous feeding damage by beetle adults in particular in late June-early July 2009 (and in 2008 at one site) allowed for a test of leafhopper response to beetle damage. The results of these tests are presented here (see Chapter 2 for a full account of the seasonal patterns of beetle and leafhopper abundances, as determined

by the censuses).

At each of the five sites, 25 tamarisk trees were marked individually to be censused repeatedly from late May to late July 2008 and mid May to early August 2009. The size of marked trees was not controlled in this study. Trees were randomly chosen at 10 meter intervals along transects in various directions at distances up 200 meters from a centrally located release tree at which *D. carinulata* beetles (~5,000 per site) had been introduced to the site in a previous year. These releases were made in 2006 at the Gateway and Saltcreek sites and in 2007 at the Bedrock and the Stan Young sites. By 2008 all sites had established beetle populations. Populations were especially small in the early summer of 2008 at Saltcreek and Stan Young sites. At Bedrock and Gateway, migrating beetles had arrived in late summer of 2007 (personal observation), likely by migrating up the Dolores River from a large population originally established in 2004 southwest of the Gateway and Bedrock sites, on the confluence of the Dolores and Colorado River in southeast Utah near Moab.

Estimating insect damage

Censuses of insect abundance and plant damage were conducted every other week from late May through late June, followed by weekly measurements from early July through early August in 2008 and 2009. The seasonal switch to weekly sampling occurred as beetle numbers increased rapidly in mid-summer at all five sites. Censuses were performed between 11:00 am and 4:00 pm. During each census, each marked tree at a site was assessed for the percent and type of foliar damage as well as for insect abundance of both specialist herbivores in adult and immature forms.

The percent foliar damage by the beetle and by the leafhopper was estimated by

examining the entire canopy of each tree to determine visually the approximate amount of damage,by each of the two herbivores as a percentage of the total tree canopy. The color of damaged foliage was used to determine whether that foliage had been damaged by *D. carinulata* or *O. stactogalus*. Adults and larvae of *D. carinulata* chew holes in the small tamarisk leaves, thereby killing more plant material than they actually consume (DeLoach et al. 2004). Foliage damaged by *D. carinulata* turns brown before eventually withering completely and falling off the branches (late in the season in most cases). In assessing beetle damage, estimates were not made of how much damaged leaf material had fallen from the tree. Instead, the percentage of foliage remaining on the tree that had turned brown was estimated. Complete defoliation of a tree by a beetle was indicated by the presence of only brown foliage throughout the tree canopy.

In contrast to foliage damaged by *D. carinulata,* foliage damaged by the phloem feeding (sucking) *O. stactogalus* appears bright yellow and stippled (such damage is especially apparent in the canopy at locations where *O. stactogalus* abundance is high). *O. stactogalus* damage tends to radiate from the center of the tamarisk canopy as leafhopper abundance on the tree grows over the season and phloem feeding increases beyond the center where the overwintering eggs initially hatch (Harding 1930). Phloem feeding damage by *O. stactogalus* is also often accompanied by the presence of sticky honeydew produced by the leafhopper (Hopkins and Carruth 1954). Removal of plant phloem along with injection of saliva into the plant wound results in typical hopperburn, or chlorosis, that is apparent on tamarisk foliage following feeding by *O. stactogalus* (Backus and Habibi 2005).

Estimating insect abundance

Beetle abundance was estimated using in situ visual estimates of total adult and larval abundance over the entire tree canopy. Three observers each carefully scanned each tree by walking around the tree and examining individual branches on the tree to estimate the approximate total abundance of adults, and of larvae, over the entire tree. The observers then reached consensus on an estimate for each tree both for adults and for larvae, and this estimate was rounded to score as belonging to one of the following six ranked categories: zero (no beetles present), one (a single individual present), two (approximately 10 individuals), three (approximately 100 individuals), four (approximately 500 individuals), five (approximately 1000 individuals) or six (approximately 1500 or more individuals).

Tap counts of four randomly chosen branches per tree were used to estimate abundance of *O. stactogalus*. A single branch in each quarter section of a tree (for a total of four branches per tree) was chosen randomly (i.e., blindly, without consideration for the extent of either beetle or leafhopper damage present). Branches varied randomly in size, with the exception that unusually large branches were excluded because they could not be sampled well by the tap count method. All branches were chosen from an approximate height of 1.5 to 2 meters on the tree. Counts of *O. stactogalus* were taken by gently shaking each branch over an upright rectangular plastic container (15 by 15 by 30 centimeters), as accomplished by tapping the branch three times on the rim of the container. The bottom of the container was fitted with a white cloth panel to increase the visibility of the very small leafhoppers once they fell from the branch into the container. After a branch had been tapped against the container rim, the *O. stactogalus* that had fallen to the bottom of the container were counted as nymphs or adults. For each tree

sampled, a single estimate of leafhopper abundance was obtained for adults, and for

nymphs, by adding together the counts for the four branches sampled.

Statistical analyses

Data were analyzed statistically by comparing the number of adult or immature *D. carinulata* or *O. stactogalus* on individual trees for a given week during the summer when the insects were at peak abundance at a given site, with the degree of foliar damage sustained previously by these individual trees from the other insect species. A two-way analysis of variance (ANOVA) was used to compare results among sites, as based on the ranks for mean numbers of *D. carinulata* (adults or larvae) in late June-early July on trees that previously during May-June had been more versus less heavily damaged by leafhoppers at individual sites (as explained in *Results* below). Trees were assigned to one or the other of the two damage categories based on the amount of damage present earlier in the season when yellow [leafhopper] damage was at its peak and could be estimated most accurately. The analysis (and all others described below) was performed using SAS (PROC GLM; SAS Institute 2010). To account for varying seasonal patterns of abundance for *D. carinulata* and *O. stactogalus* among sites, results (for estimates of leafhopper damage and of peak abundances of beetle adults and larvae) from different census dates were used in the analysis for the two more southerly sites (Gateway and Bedrock) versus the more northerly sites (Saltcreek and Stan Young) (Table 3.1). A similar analysis was not conducted for 2009 because very few leafhoppers and very little leafhopper damage occurred at any of the five study sites early in the season of 2009.

Two-way ANOVAs were also performed to compare results among sites (in a given year) or years (for a given site) based on the mean number of *O. stactogalus* (adults or

nymphs) in mid-summer on trees that had previously been more versus less heavily damaged by *D. carinulata* (as measured earlier in the season when brown [beetle] damage was at its peak and could be estimated most accurately). In 2008, sufficient beetle damage for analysis occurred only at one site (Gateway). For this site in 2008 and 2009, a two-way analysis of variance (year x damage level) was based on *D. carinulata* damage and *O. stactogalus* counts as measured on dates given in Table 3.2. Sufficient *D. carinulata* damage occurred at two other sites (Saltcreek and Stan Young Burned) in 2009 to enable a two-way analysis of *O. stactogalus* responses (to varying *D. carinulata* damage x site; see table 3.2 for census dates used in the analysis).

To test whether any observed differences in numbers of second generation *O. stactogalus* on trees with light (0%-25%) versus heavier (26%-100%) damage by *D. carinulata* might be attributable in part to abundances of first generation leafhoppers on the trees before peak beetle damage occurred, an additional analysis was conducted. The numbers of *O. stactogalus* individuals on trees with low versus higher foliar damage by beetles were compared at the first census date early in the season when at least five marked trees had incurred 50% or greater beetle damage, as indicated in Table 3.2.

Beetle host choice and development experiments

Testing for beetle host choice was performed within a large screened cage (4 meters by 4 meters by 2.5 meters tall) built on the grounds of the Colorado Department of Agriculture Palisade Insectary at Palisade, Colorado. Host selection of *D. carinulata* adults was compared within the cage by simultaneously offering the adults individual tamarisk plants that either were undamaged or had been damaged previously by *O. stactogalus* feeding so that 50-75% of the foliage had turned yellow.

For the experiment, potted tamarisk plants were reared in the Insectary greenhouse using cuttings collected in March 2008 from a single tree located in the McGinnis Conservation area in Horsethief Canyon, Colorado. Once the plants reached approximately one meter in height, fine mesh sleeves were placed over each of half of the potted plants, followed by the addition of ~150 *O. stactogalus* adults plus a few late instar nymphs. These leafhoppers had been collected from tamarisk growing on the grounds of the Palisade Insectary. The leafhoppers were left on the tamarisk plants for approximately four weeks, resulting in the desired 50-75% total foliar damage from phloem feeding. Tamarisk damage was scored by the appearance of yellow foliage and the presence of honeydew indicative of feeding by *O. stactogalus*. Once the desired level of damage had been reached on all potted treatment plants, the *O. stactogalus* were removed by gently tapping them from individual branches on the potted tamarisk plants. Because *O. stactogalus* were removed from all plants at the same time, damage varied among the potted plants, but the total percent foliar damage for each plant fell within the range of 50-75%.

Eight plants without damage were paired with eight plants that had been damaged by *O. stactogalus*, for a total of 16 potted tamarisk plants. Two pairs of plants (i.e., four plants) were used in each of four replications of the experiment. In each replication, damaged and control plants were placed in alternating corners of the cage. Approximately 200 *D. carinulata* adults, that had been collected from tamarisk 16 kilometers east of the Gateway site near Gateway, Colorado, were released in the center of the cage on the afternoon of 14 August 2008. Upon collection (on 12 August 2008), the *D. carinulata* were held at natural daylength in a cage within an isolation chamber in

the Palisade Insectary for two days prior to release in the large experimental cage. During these two days, the *D. carinulata* were fed daily with tamarisk bouquets made from tamarisk growing on the grounds of the Palisade Insectary.

At the start of the experiment, the *D. carinulata* were released onto the screened floor in the center of the large experimental cage at approximately 1:00 pm. This release time was chosen to encourage flight and settling onto test plants immediately upon release (the beetles naturally are most active at mid day). For the next six hours, the *D. carinulata* were monitored at hourly intervals for their activity and locations. *D. carinulata* abundance on each plant was measured at each hourly check by counting the number of adults observed by visually scanning the entire plant. An index of *D. carinulata* use of control versus treatment plants during the six-hour observation period was determined by summing the hourly *D. carinulata* counts for the two plants (damaged or control) combined. The number of *D. carinulata* that were found on the inner cage walls was also recorded at each hourly check. The morning after each experimental replication, the adults were collected and removed from the cage. A new set of undamaged and damaged plants was placed in the cage in the morning, followed by a new group of approximately 200 adult *D. carinulata* at 1:00 pm, collected at the same time and place as the first group, and the experiment was repeated as described above. In total, four experimental trials were performed over four consecutive days (14-17 August). The weather was consistently warm and dry, with a mid day average temperature of ~34 C° throughout the time period of the experiment. The index of *D. carinulata* abundance for damaged versus undamaged potted tamarisk was compared across the four trials using a paired t-test, performed in SAS (PROC TTEST; SAS Institute 2010).

In a second caged beetle experiment, rates of pupation and adult emergence for *D. carinulata* were compared for larvae placed on potted tamarisk plants that were either undamaged or had been damaged previously by *O. stactogalus*. Twenty potted plants were grown from cuttings collected from a single tree in the McGinnis Conservation area at Horsethief Canyon, in March 2009. Once the plants reached approximately one meter in height mesh sleeves were placed over the plants, and approximately 100 *O. stactogalus* adults plus about half as many late instar nymphs, were added to each of half of the caged plants. The leafhoppers were collected from tamarisk growing on the grounds of the Palisade Insectary. The *O. stactogalus* were added for roughly four weeks beginning on 5 July 2009 to achieve 50-60% total foliar damage resulting from phloem feeding. Tamarisk damage was scored by the appearance of yellow foliage and the presence of honeydew indicative of feeding by *O. stactogalus*. Once the desired level of damage (i.e., 50-60% yellow foliage) had been reached on all potted treatment plants, the leafhoppers were removed by gently tapping them from individual branches on the potted tamarisk plants. Five of the damaged plants were also rinsed of honeydew by gently submerging the foliage on each branch in water for 30-60 seconds to produce a branch that no longer felt sticky. Because all of the leafhoppers and the honeydew from five of the damaged plants were removed at the same time, damage varied among plants on which leafhoppers had fed, but the total percent foliar damage fell within the range of 50-60% with honeydew (when not removed) covering at least half of the total foliage surface.

Twenty-five *D. carinulata* 1st instars were then added to each of the ten sleeved and damaged plants, and also to each of five, randomly chosen plants that had been

sleeved without leafhoppers added (i.e., undamaged plants). The larvae were obtained

from eggs produced by caged *D. carinulata* that had been collected approximately 16

kilometers south of the Gateway site and held under full day length conditions for two

weeks while producing eggs. Tamarisk bouquets were placed with the eggs daily until

they hatched, at which time hungry first instars climbed onto foliage and were collected

to place on sleeved plants. Each sleeved plant received ten first instars on 20 July 2009,

and fifteen more the following day (for a total of 25 first instars per plant). These larvae

were monitored visually for growth and survival each day until they dropped from the

foliage to pupate in the soil. Fully mature larvae that had dropped from the foliage were

collected daily and placed together in small deli containers with an approximate

circumference of 10 cm and a height of 6 cm. These containers had been filled with sand

to a depth of 3 centimeters. The larvae pupated in the sand and were held until adult

emergence. The containers were held in an incubation chamber set at full daylength

conditions or a photoperiod 16:8 (L:D) and 25 $^{\circ}$C. Proportions of larvae that pupated and

that ultimately emerged as adults were compared among treatments by using one-way

ANOVA with linear contrasts: first, proportions were compared between undamaged and

damaged plants, and then proportions were compared between damaged plants that were

unwashed or that had been washed to remove leafhopper honeydew.

Leafhopper host choice experiment

A leafhopper host choice test was performed using a large screened cage (1 meter

by 1 meter by 1.5 meters tall) built on the grounds of the Palisade Insectary. The host

selection of leafhoppers was compared between tamarisk plants with ~50% *D. carinulata*

damage versus undamaged tamarisk plants. Potted tamarisk plants were reared using 16

cuttings collected from a single tree located in McGinnis Conservation area located in Horsetheif Canyon, in March of 2009. Once the plants reached approximately one meter in height, fine mesh sleeves were placed over the potted plants and ~20 *D. carinulata* adult*s* were added to half of the plants and allowed to feed and lay eggs for one week. Beetles were added on the same day that they were collected from tamarisk 2 kilometers east of the Gateway site near Gateway, CO. After one week, the adults were removed and the experimental plants remained sleeved for approximately two more weeks to allow egg eclosion and larval development that resulted in approximately ~50% defoliation. The larvae were then removed. The ten damaged plants were paired with ten undamaged (control) plants, for a total of 20 potted tamarisk plants that were used in groups of four for five trials of the experiment. Each trial consisted of the placement of two damaged plants and two control plants in alternating corners of the cage. Approximately one hundred *O. stactogalus* adults and a far lesser amount of nymphs (~50) were collected from the grounds of the Palisade Insectary for each trial and were released in the center of the cage at 9:00 am (beginning on 27 July 2009 for the first trial).

Monitoring began the following day at 9:00 am and was repeated at this time over the next three days for a total of four measurements per trial of *O. stactogalus* abundance on damaged versus undamaged plants in each experimental run. Adult *O. stactogalus* abundance was counted in situ with close examination of each plant for ten minutes per potted plant. At the conclusion of each trial, potted plants and *O. stactogalus* were removed and a new set of plants and *O. stactogalus* were added to the cage. Thus, five experimental replications were performed over 20 consecutive days (28 July - 17 August). The weather was consistently warm at mid day (~34.3 ^{o}C) and dry throughout

the time period of the experiment. A paired t-test was performed in SAS (PROC TTEST; SAS Institute 2010) to compare an index of *O. stactogalus* abundance (i.e. the sum of leafhopper abundance over the four-day sampling period) on damaged versus undamaged plants within each trial.

Results

Beetle response to leafhopper damage

Almost no early season phloem feeding damage by *O. stactogalus* was observed at the Stan Young unburned site in 2008, so beetle response to leafhopper damage could not be evaluated at this site. At the other four sites in 2008, early season phloem feeding damage by *O. stactogalus* was also light or absent on many trees, and never exceeded 25% on individual trees at any of the sites. However, the mean extent of yellow damage steadily increased at the four sites during June as the first generation of leafhoppers matured, while feeding damage from beetles (brown damage) remained light. Both adult and larval *D. carinulata* abundances in July 2008 were lower on trees moderately damaged by the leafhopper versus on undamaged or lightly damaged trees (i.e., 5-25% versus 0-1% foliar damage; Figure 3.1). This pattern was observed consistently at each of the four sites despite the large variation in the numbers of beetles present at individual sites (Figure 3.1). Because of high variance in beetle abundance among trees, however, the overall trend was not statistically significant in two-way ANOVA for either beetle adults or larvae (Table 3.3). Even so, in the absence of any differential response of beetles to trees of varying leafhopper damage, the two-tailed probability that observed abundances of beetles (adults or larvae) would be greater by chance alone at all four sites on trees with slight versus moderate damage is only 0.06 (i.e., $[1/2]^4$).

Slightly more adults of *D. carinulata* were found on undamaged individuals of *T. ramosissima* versus individuals that had been damaged by *O. stactogalus* in the large cage experiment but the difference was not significant (Figure 3.2; paired t-test; t = 1.77, DF = 3, P = 0.1749). A large proportion of beetles (~150 individuals) did not choose among plants during the test period, and instead were found on the cage walls. Thus the experiment was only moderately successful in creating conditions conducive to the aggregation behavior that is typical of these beetles as they choose host plants in natural settings.

In the second (small cage) experiment, beetle larvae that were placed on *O. stactogalus* damaged tamarisk (either unwashed or washed to remove leafhopper honeydew) did not show a strong tendency to feed on green, undamaged foliage rather than yellow foliage. However, the presence of honeydew appeared to slow the movement of the larvae. The proportions of *D. carinulata* larvae that pupated, and of pupae from which adults emerged, were slightly but non-significantly reduced when the beetles had been grown on *O. stactogalus* damaged tamarisk versus undamaged tamarisk (Figure 3.3 and Table 3.4). The strongest difference observed in this experiment occurred between pupal performance associated with larvae grown on washed versus unwashed plant material that had been damaged by leafhoppers. The presence of sticky honeydew on unwashed plant material reduced adult emergence to almost half the rate of adult emergence when larvae were grown on undamaged tamarisk plants (Figure 3.3). This difference, however, was not statistically significant (Table 3.4).

Leafhopper response to beetle damage

Accumulated damage by the first generation of *D. carinulata* was absent or only very slight among trees at four of the five study sites early in the season in 2008. Only at the Gateway site did appreciable foliar damage (greater than 25%) occur. At this site in 2008, leafhopper adults occurred in greater numbers in July on trees that previously had been lightly damaged (0-25%) versus more heavily damaged (26-100%) by beetles (Figure 3.4). Although far fewer leafhopper adults were found at this site the next year (2009), the same pattern in their numbers on individual trees in response to previous beetle damage (i.e., early season damage as had accumulated by mid-June 2009) was apparent (Figure 3.4 and Table 3.8). In contrast, comparison of leafhopper abundances on trees prior to peak damage by the beetle revealed no difference in numbers of leafhoppers on trees that were subsequently lightly versus more heavily attacked by beetles (Table 3.7 and 3.8). Nymphs were not found in sufficient numbers at the Gateway site in either year for inclusion in analysis.

In addition to trees at Gateway, trees at two other sites, Saltcreek and Stan Young Burned, received significant damage from first generation beetles in 2009. In contrast to damage at the Gateway site, damage was never complete (i.e., greater than 90%) on individual trees, and very rarely reached above 40% for individual trees at either of these sites. Leafhopper adults in mid- July were somewhat more abundant on trees with light versus heavier beetle damage at these sites (Figure 3.4), but the difference was not as marked as that at Gateway, and was not statistically significant (Table 3.9). More leafhopper nymphs occurred at these two sites than at Gateway, and these nymphs occurred in significantly greater numbers on trees that were less heavily damaged (Figure 3.4 and Table 3.9).

When leafhoppers were provided with both damaged and undamaged host plants in the caged host choice test, a significantly greater number of adult leafhoppers chose undamaged *T. ramosissima* (Figure 3.6; paired t-test; t = 2.80, F = 4, P = 0.0486). Although the *O. stactogalus* were very hard to see on the plants, it appeared that they moved very little from the host plants that they chose at the beginning of the experiment (i.e., *O. stactogalus* counts varied little over the four day sampling period). *O. stactogalus* adults were rarely seen on the *D. carinulata* damaged portions of the treatment plants and were mostly observed on green plant material among both damaged and undamaged potted *T. ramosissima*. *O. stactogalus* adults were also observed to occur in close proximity to one another while feeding. Although nymphs were observed among the control and treatment plants, the difficulty of observing them in situ resulted in excluding them from analysis.

Conclusions

The field observations and the results of cage experiments, when taken together, suggest that *D. carinulata* and *O. stactogalus* do not interact with each other positively through facilitation, such that host plant damage by one species leads to greater damage from the other species as well. Instead, the results suggest that these two herbivore species interact with each other in negative, competitive fashion in sharing tamarisk as a host plant, such that feeding damage from one species leads to reduced attack by the second species. As discussed below, this is most apparent in the present study for the response of the leafhopper to prior beetle damage to tamarisk.

A number of field studies have examined plant-mediated competition and shown that early season herbivory can and does affect subsequent colonizing species including

mites, microlepidoptera, planthoppers, and beetles (English-Loeb et al. 1993, Karban

1993, Denno et al. 2000, Viswanathan et al. 2005). The recent renewal of interest in

insect competition as mediated by plant response is important for further refinement of

the ecological roles and interactions of a variety of herbivorous insects. In particular, it is

important in the field of biocontrol, specifically as it bears on the release of biocontrol

insects at sites where prior herbivory by other specialist herbivores has occurred. In the

case of tamarisk, it is of issue whether the presence of the leafhopper affects the success

of future beetle releases.

Plant-mediated competition among herbivores could arise from simple exploitative

interaction by consumption of plant tissue. It may also arise as the result of induced plant

defenses. The presence of a strong, induced plant defense following phloem feeding by

the leafhopper could negatively affect future establishment of the beetle as a biocontrol

agent for tamarisk. The results of this study, however, provide little support that a strong,

induced plant defense by *T. ramosissima* following phloem feeding by *O. stactogalus*

occurs and affects subsequent host use by *D. carinulata*. In neither field observations nor

cage experiments did the beetles respond strongly to tamarisk trees that were undamaged

versus damaged previously by the leafhopper. The presence of leafhopper damage did

reduce host selection by adult beetles somewhat at the field sites, but this influence was

slight and insufficient for producing significant, major differences subsequently in larval

abundance among undamaged and damaged trees. Similarly, cage tests produced some

indications of reduced fitness when larvae fed on tamarisk plants damaged previously by

the leafhopper, but such reduction in fitness seemed more tied to the presence of

leafhopper honeydew on the tamarisk foliage than to any alterations in plant physiology

and host tissue quality from the presence of leafhopper herbivory.

Many sap-feeders are known to cause only minimal tissue damage to the plants on which they feed, therefore acting as "stealthy" herbivores (Raven 1983, Heidel and Baldwin 2004, Kaplan and Denno 2007). The particular feeding style of *O. stactogalus* may minimize its effects on foliage quality for *D. carinulata*. This leafhopper species is thought to penetrate plant tissue deeply (relative to the insect's body size) because it extends its maxillary stylets beyond the tip of the labium (Snodgrass 1935, Wiesenborn 2004). This style of feeding is associated with plant injury that is confined to the individual cells into which the stylets penetrate, perhaps thereby reducing overall plant injury (Wheeler 2001). Although reduced growth of tamarisk has been measured in previous studies, leafhopper abundances must be very large to cause substantial reduction in plant growth (Liesner 1971). At lower abundances, leafhoppers are likely to only extract limited quantities of carbohydrates and other nutrients, which could lead to modest reductions in growth of the plant and perhaps more importantly, little likelihood of greatly reduced foliage quality for the beetle (Liesner 1971, Wiesenborn 2004).

The muted response of the beetle to previous leafhopper damage in the field observations reported here may reflect not only limited effects of leafhopper damage on the nutrient content of tamarisk foliage, but also the relatively low amounts of honeydew that occurred at the field sites during the study. In the field, tamarisk may become greatly contaminated with honeydew if conditions and resources are adequate to allow leafhoppers to increase rapidly in abundance early in the season, but more typically, leafhopper abundances do not build to reach high numbers until mid season (Harding 1930). When the foliage is greatly contaminated with honeydew, this may impair

movement of larvae (as observed in field cages) and thereby affect growth and survival, but during the present study, foliage heavily contaminated with honeydew was rarely seen in the field until later in the season (i.e. mid to late July). Such seasonal timing makes it unlikely that leafhopper honeydew deposition will often strongly affect host choice and larval performance of the beetle in the field.

One interesting consequence of leafhopper honeydew deposition on tamarisk foliage, however, is its attraction of harvester ants, predators of beetle larvae (personal observation). The effect of this on beetle survivorship was not measured by this study, but it is a form of indirect (top down) competition that may reduce the beetle's fitness when it feeds on leafhopper damaged plant material. The presence of honeydew and subsequent ant foraging could have particular ramifications on late season establishment of the beetle. This type of indirect competition has been well documented in other study systems of insect herbivores and their natural enemies (Evans and England 1996, Kaplan and Denno 2007, Styrsky and Eubanks 2007, Ohgushi 2008).

In contrast to the somewhat weak response of the beetle to previous herbivory by the leafhopper, the response of the leafhopper to previous damage from the beetle was much stronger. This suggests an asymmetric competitive effect as produced by prior beetle herbivory. Reductions in availability of suitable plant tissue to support phloem feeding by the leafhopper likely led to significant decreases in adult leafhopper abundance on trees at the Gateway site where damage by the beetle was greatest. The cage experiment also revealed that the leafhoppers select undamaged stems on which to feed when the herbivore occurs on a tamarisk plant previously damaged by beetles. Although adult leafhopper abundances were not reduced significantly in beetle damaged

trees at the Stan Young and Saltcreek Sites in 2009, beetle damage was also substantially lower at these sites, perhaps lessening the effect of reduced foliage quantity and quality for phloem feeding by the leafhopper. An effect was more apparent in abundances of leafhopper nymphs, which were reduced on beetle-damaged versus undamaged trees at all three sites in 2009 as well as at the Gateway site in 2008. Trees with relatively heavy damage from beetles had supported similar or even greater numbers of first generation leafhoppers earlier in the season that had trees with light beetle damage. Cage testing also indicated that leafhoppers occurred in much smaller numbers on tamarisk trees that had been damaged previously by beetles than on undamaged trees.

It might be possible that leaf feeding by the beetle produced an induced plant response in tamarisk that resulted in the altered patterns of abundance of the leafhopper seen in the field and cage tests. Leaf feeding beetles have been shown to elicit changes in secondary metabolite expression resulting in the reduced fitness of co-occuring herbivores, and the response of the leafhopper could be due at least in part to reductions in food quality or physiological changes as well as food quantity, as the host plant sustains extensive feeding damage by the beetle (Kaplan and Denno 2007). This may be the case here. However, the major effect of beetles on leafhoppers may arise from the great amount of damage to foliage that the beetles often inflict on the host plant. In large numbers these beetles have been recorded to defoliate hundreds to thousands of acres of tamarisk, an occurrence which likely results in mass reductions of leafhopper abundance or at least great displacement of the leafhopper into areas without great beetle damage (DeLoach et al. 2004). Although the leafhopper was found at the Gateway site in 2009

following complete defoliation of tamarisk by the beetle in 2008, the decrease in measured abundance in first generation leafhoppers from 2008 to 2009 was nearly 95%.

The results of this study suggest that even when leafhopper abundance is high, damage by this herbivore to tamarisk early in the season has relatively little effect in altering the fitness and subsequent host choice of the beetle. Thus the leafhopper is unlikely to hinder the establishment of the beetle as a biocontrol agent. The effect of the beetle on the leafhopper is much stronger. The leafhopper, however, is not considered a major biocontrol agent for tamarisk. Although the leafhopper is capable of damaging tamarisk, its effects on the host plant appear far less damaging than those of the beetle. Thus reductions in leafhopper abundance that are caused by beetle herbivory are more of general ecological interest, rather than of special concern for the successful biological control of tamarisk.

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Table 3.1. Dates in 2008 on which early-season *O. stactogalus* herbivory was measured, and on which abundances of *D. carinulata* adults and larvae were measured, for comparing beetle abundance with previous leafhopper damage.

Site	Year	Leafhopper Damage	Adult Abundance	Larval Abundance
Gateway	2008	June 22	July 1	July 8
Bedrock	2008	July 1	July 10	July 17
Saltcreek	2008	July 9	July 16	July 29
Stan Young Burned	2008	July 9	July 18	July 31

Table 3.2. Dates in 2008 and 2009 on which early-season *D. carinulata* herbivory was measured, and on which abundances of *O. stactogalus* adults and nymphs were measured, for comparing leafhopper abundance with previous beetle damage (nymphal abundances were too low for inclusion in analysis at the Gateway site).

Site	Year	Beetle Damage	Adult Abundance	Nymphal Abundance
Gateway	2008	July 1	July 8	
Gateway	2009	June 17	June 22	
Saltcreek	2009	July 8	July 16	August 14
Stan Young Burned	2009	July 9	July 15	August 11

Table 3.3. Results of two-way analyses of variance for the effect of early season leafhopper damage and site on the numbers of beetle adults and larvae that occurred subsequently on tamarisk trees at Saltcreek, Gateway, Bedrock and SY Burned in 2008 (P values shown are two-tailed for the effect of damage).

Across Site Response	Source	Df	MS	F	P
Adult response	Damage	1	1	2.46	0.12
	Site	3	1.60	3.48	0.02
	Damage x site	3	0.13	0.28	0.82
	Error	92	0.46		
Larval response	Damage	1	0.31	0.23	0.64
	Site	3	11.03	8.37	<0.0001
	Damage x site	3	0.42	0.32	0.82
	Error	92	1.32		

Table 3.4. Results of one-way ANOVA with linear contrast to assess the effects of host plant quality on *D. carinulata* larval pupation and adult emergence when larvae fed on undamaged (control) host plants (*T. ramosissima*) versus on host plants that had been damaged by *O. stactogalus* feeding, with or without leafhopper honeydew removed (yellow damage versus yellow + sticky damage).

Source	Df	MS	F	P
Treatment Effect on Pupation				
Damage vs. Control	1	8.53	0.29	0.60
Yellow vs. Yellow + Sticky Damage	1	10.00	0.34	0.57
Error	12	353.20	29.43	
Treatment Effect on Emergence				
Damage vs. Control	1	20.83	2.03	0.18
Yellow vs. Yellow + Sticky Damage	1	28.90	2.81	0.12
Error	12	123.20	10.27	

Table 3.5. Results of two-way analyses of variance for the effect of early season beetle damage and site on the numbers of leafhopper adults and nymphs that occurred subsequently on tamarisk trees at Gateway in 2008 and 2009 (P values shown are two-tailed for the effect of damage).

Southern Sites	Source	Df	MS	F	P
Adult response	Damage	1	48.69	8.63	<0.0001
	Year	1	187.1	33.32	<0.0001
	Damage x site	1	20.77	3.68	0.06
	Error	1	5.64		

Table 3.6. Results of two-way analyses of variance for the effect of early season beetle damage and site on the numbers of leafhopper adults and nymphs that occurred subsequently on tamarisk trees at Saltcreek and SY Burned in 2009 (P values shown are two-tailed for the effect of damage).

Northern Sites	Source	Df	MS	F	P
Adult response	Damage	1	1	0.44	0.50
	Site	1	7.46	4.92	0.04
	Damage x site	1	0.00	0.00	0.88
	Error	41	1.51		
Nymphal response	Damage	1	16.96	6.65	<0.0001
	Site	1	24.55	9.63	<0.0001
	Damage x site	1	8.82	3.46	0.06
	Error	41	2.55		

Table 3.7. Results of two-way analyses of variance for the effect of trees previous to lighter and heavier beetle damage and site on the numbers of early season leafhopper adults that occurred on tamarisk trees previous to peak beetle damage at Gateway in 2008 and 2009 (P values shown are two-tailed for the effect of damage).

Gateway 2008 and 2009	Source	df	MS	F	P
Adult response	Damage	1	0.152	0.01	0.9158
	Year	1	467.48	34.71	<0.0001
	Damage x year	1	32.42	2.41	0.1280
	Error	1	13.47		

Table 3.8. Results of two-way analyses of variance for the effect of trees previous to lighter and heavier beetle damage and site on the numbers of early season leafhopper adults and nymphs that occurred on tamarisk trees previous to peak beetle damage at Saltcreek and SY Burned in 2009 (P values shown are two-tailed for the effect of damage).

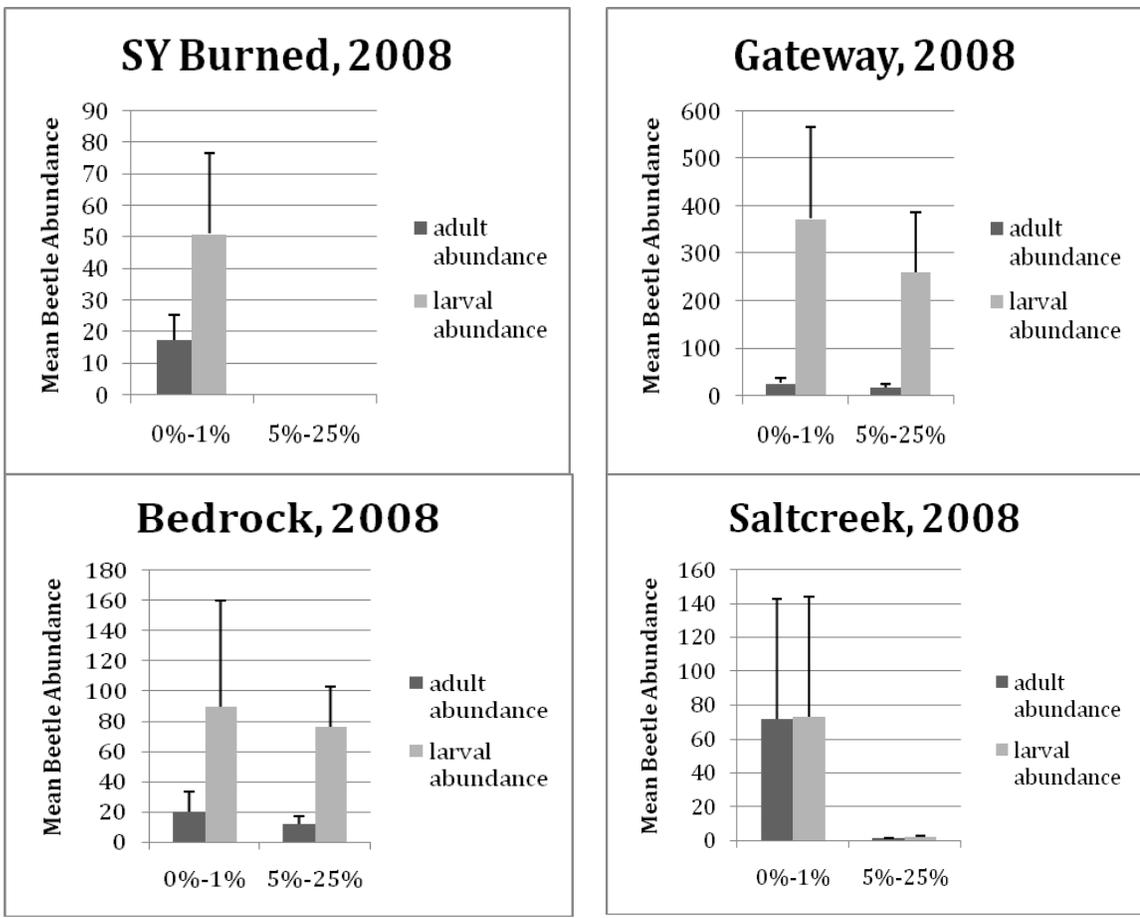| Saltcreek and Stan Young 2009	Source	df	MS	F	P
Adult response	Damage	1	0.25	0.13	0.72
	Site	1	0.06	0.03	0.86
	Damage x site	1	0.50	0.26	0.61
	Error	42	1.94		
Nymphal response	Damage	1	30.13	2.25	0.14
	Site	1	20.23	1.51	0.23
	Damage x site	1	0.00	0.00	1.00
	Error	42	13.40		

Figure 3.1. The mean number (+ one standard error) of adults and larvae of the tamarisk beetle *D. carinulata* that occurred on host trees that were damaged lightly or more heavily (i.e., (0%-1% versus 5%-25% of the canopy damaged) earlier in the season in 2008 by *O. stactogalus* herbivory.
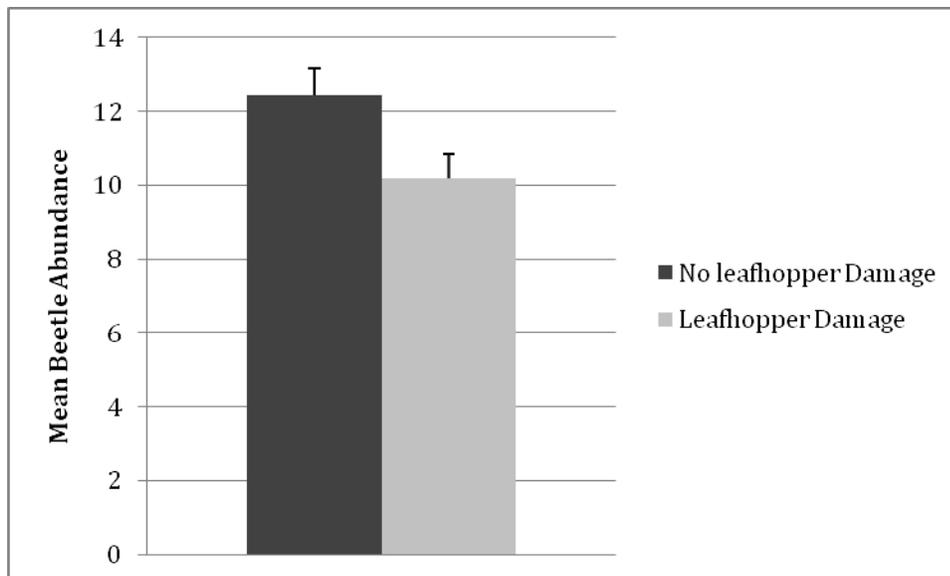
Figure 3.2. Mean *D. carinulata* abundance (+ one standard error) on undamaged individuals of *T. ramosissima* versus individuals that had been damaged previously by *O. stactogolus,* as measured in the large cage experiment.
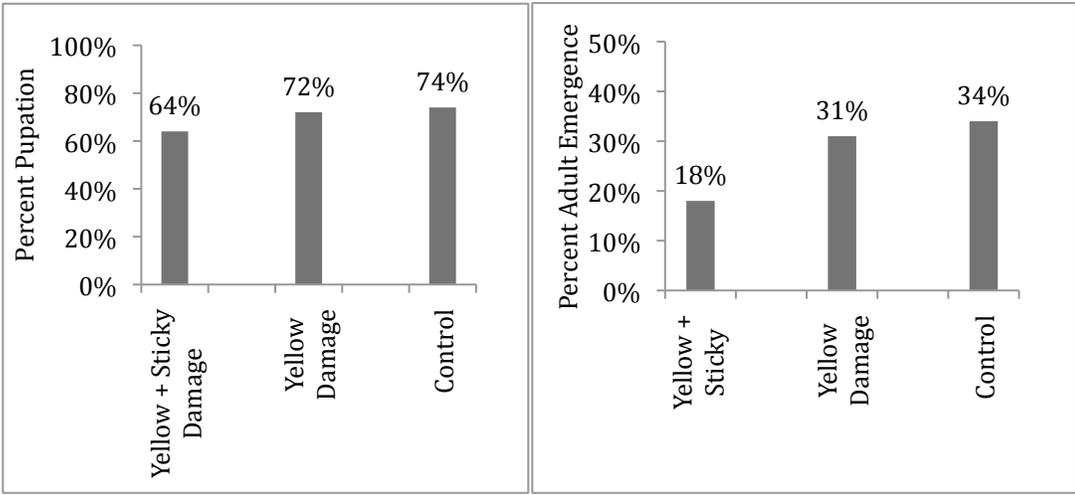
Figure 3.3. The percentage of tamarisk beetle larvae that pupated, and the percentage of these larvae that emerged from pupae as adults, when larvae fed on undamaged (control) host plants (*T. ramosissima*) versus on host plants that had been damaged by *O. stactogalus* feeding, with or without leafhopper honeydew removed (yellow damage versus yellow + sticky damage
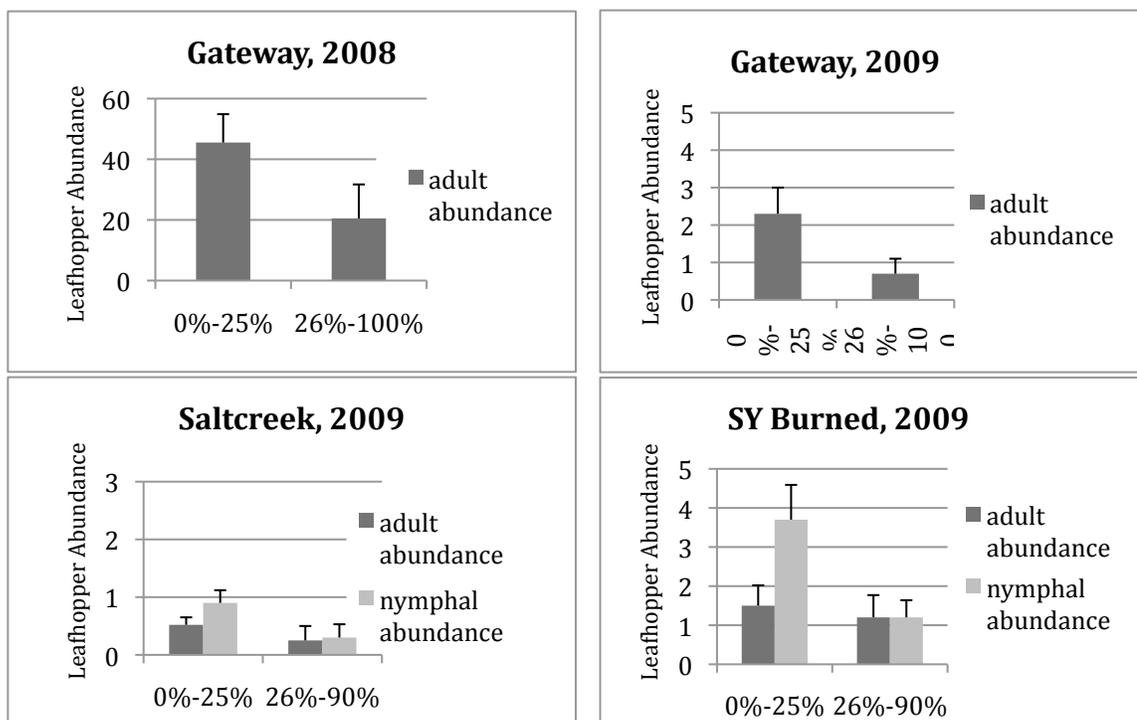
Figure 3.4. The mean number (+ one standard error) of adults and nymphs of the leafhopper *O. stactogalus* that occurred on host trees that were damaged lightly or more heavily (i.e., (0%-25%.versus 26%-100% of the canopy damaged) earlier in the season by *D. carinulata* herbivory.
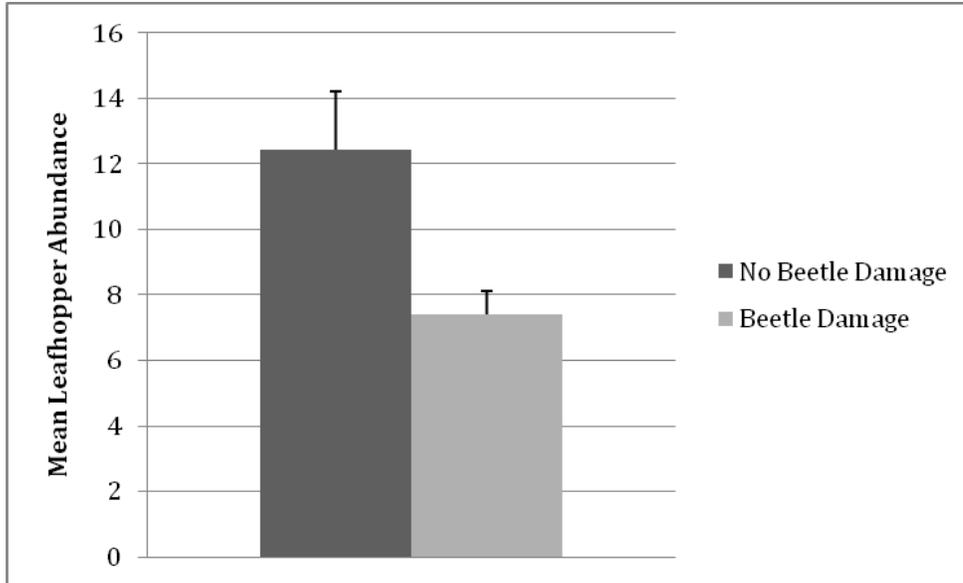
Figure 3.5. Mean *O. stactogalus* abundance on *D. carinulata* damaged vs. undamaged potted *T. ramosissima* in a caged setting.

CHAPTER 4

CONCLUSIONS

The successful establishment and subsequent defoliation of tamarisk, *Tamarix ramosissima,* by the tamarisk beetle, *Diorhabda carinulata*, across several monitored sites in western Colorado, as documented in this study, further confirms the ability of this beetle to damage and defoliate the plant. As confirmed also in this study, the beetle shares the host plant with an abundant, second species of specialist herbivore, the tamarisk leafhopper, *O. stactogalus*. The leafhopper was found consistently throughout the growing season at all field sites in the present study, with phenological patterns that overlapped and interdigitated with the phenological patterns of the beetle.

The present study demonstrates the ability of the tamarisk beetle to damage and defoliate tamarisk despite pre-existing damage by the tamarisk leafhopper. This study also adds supporting evidence that *T. ramosissima* exhibits little or no plant defense following herbivory by *O. stactogalus*. There is a possibility that an induced plant defense following herbivory by *D. carinulata* resulted in the altered abundances of *O. stactogalus* that were shown in field and in cage tests; however, these changes in leafhopper abundance may have also resulted from limited plant material remaining after beetle damage. In either case, such changes in abundances of the leafhopper would appear to have little consequence for the overall level of damage to tamarisk. In the absence of strong negative effects of the leafhopper on the beetle, the continued success of *D. carinulata* as an introduced biocontrol agent for tamarisk seems assured even at sites where *O. stactogalus* has previously established. The long-term consequence for biological control of the beetle's effect on the leafhopper as mediated by feeding on

tamarisk is less clear, but the interaction is nonetheless of interest from an ecological perspective.

Although the results of this study suggest that *T. ramosissima* is not capable of defending itself against attack by the tamarisk beetle following injury by the tamarisk leafhopper*,* the beetle did show signs of some host preference among damaged and undamaged tamarisk. The beetle appeared to favor trees with little or no previous damage by the leafhopper. Although high variance in beetle abundances among damaged and undamaged trees occurred at field sites in this study, the same host choice pattern (greater abundance on trees least damaged by leafhoppers) was apparent at all sites, resulting in a strongly suggestive trend. A cage test also showed a slight yet non-significant trend in the host choice of the beetle adults for undamaged potted tamarisk rather than tamarisk previously damaged by *O. stactogalus*. A second cage test similarly suggested reduced performance of immature beetles when grown on *O. stactogalus* damaged versus undamaged tamarisk. The tendency to prefer and perform better on undamaged tamarisk foliage may have been due in part to the presence on damaged foliage of honeydew excreted by the leafhopper as it feeds. The preference of beetle adults may also arise because some individuals (although field censuses suggest this would include relatively few adults) may respond to reduced plant tissue quality as caused by the previous herbivory of the leafhopper.

The much stronger negative response of the leafhopper to prior damage from beetle herbivory found in this study could involve an induced plant defense. However, an induced defense cannot be confirmed by this study. The wilting and subsequent defoliation of tamarisk leaves and stems as caused by beetle herbivory ultimately

removes plant material for the leafhopper to feed on, and thus limits the physical space on

the host plant that the leafhopper can inhabit. This removal of plant material for the

leafhopper to feed and lay eggs may account fully for leafhopper responses to beetle

damage as observed in this study, regardless of any physiological changes the plant may

undergo.

Although the true cause of altered leafhopper abundance as seen in this study is

hard to disentangle, the response of the leafhopper to previous beetle damage is helpful in

documenting that an asymmetric competitive interaction exists between these two

specialist herbivores in their introduced range. In contrast, this study provides little

support for a facilitative interaction between these specialist herbivores: neither of these

specialist herbivores responded positively to interspecific plant damage, as I had initially

hypothesized. I had hypothesized that the interactions between these species would be a

combination of both facilitative and competitive effects, but my hypotheses were not

supported by this study. Prior damage to tamarisk by *O. stactogalus* did not strongly deter

feeding by *D. carinulata,* resulting in lower beetle defoliation rates on tamarisk trees with

high *O. stactogalus* abundance and damage. Instead there was little to no competitive

effect of the leafhopper on the beetle. I also had predicted that defoliation by *D.*

carinulata would increase host attractiveness for *O. stactogalus*, and consequently greater

O. stactogalus abundances would be found on tamarisk trees with previous damage by *D.*

carinulata. Instead there was no facilitative effect of the beetle on the leafhopper, but

rather a competitive effect. Thus *O. stactogalus* were not found in greater abundances

following damage to tamarisk by *D. carinulata*. Instead the leafhopper abundance was

reduced on trees that previously had been damaged moderately (versus only lightly) by the beetle.

Future work should focus on determining in detail why numbers of *O. stactogalus* among individual trees and among sites vary within and among years. The slight, yet consistent trend among sites of the tamarisk beetle to discriminate between *O. stactogalus* damaged and undamaged tamarisk is also worthy of further investigation. For instance, are *T. ramosissima* populations in North America under selective pressure to develop an induced defense to insect herbivory, a response that has not yet become strong enough to deter the beetle? Perhaps interactions of the plant with the beetle and leafhopper in tamarisk's native range could provide important clues as to the evolved responses of all three species that have arisen as they have persisted together over the long-term. I suspect that the interaction of these species in their introduced range may reach an equilibrium similar to what may occur in the native range, as these species adjust their responses to one another over time in the new circumstances of the invaded range. Future studies investigating the potential for change in the responses and interactions of these species would be quite interesting and important for broadening our understanding of the nature of interactions of introduced species as they naturalize outside of their native range.