DISSERTATION

THE ECOLOGY AND EVOLUTION OF PLANT-INSECT INTERACTIONS AMONG HYBRID POPULATIONS OF THE INVASIVE PLANT, TAMARISK (*TAMARIX* SP.), IN THE WESTERN UNITED STATES

Submitted by

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ABSTRACT

THE ECOLOGY AND EVOLUTION OF PLANT-INSECT INTERACTIONS AMONG HYBRID POPULATIONS OF THE INVASIVE PLANT, TAMARISK (*TAMARIX* SP.), IN THE WESTERN UNITED STATES

Tamarisk is one of the most abundant invasive tree species in the western United States. Several species belonging to the genus *Tamarix* were imported intentionally to the U.S. in the mid-nineteenth century. Currently, most U.S. populations are comprised of a hybrid swarm between *T. ramosissima* and *T. chinensis* and other species. Negative consequences of hybrid tamarisk invasion include alteration of ecosystem functioning and decreases in native biodiversity. Very few natural enemies attack this invasive plant, contributing to its success on the landscape. In an attempt to provide top-down population control, a specialized herbivore that coevolved with tamarisk in its native range was intentionally released in the introduced range (i.e. biological control). I investigated interactions between tamarisk hybrids and herbivores in order to better understand the dynamics that contribute to the control of this exotic weed. In Chapter 1, which was published in Volume 57 of The Southwestern Naturalist, I describe how a native stem-boring beetle was found attacking tamarisk populations in eastern Colorado, western Kansas, and southwest Nebraska. This is an important discovery because very few native insects have been reported to consume this plant and never at the levels of the stem-borer. The beetle may reduce tamarisk growth and fecundity on the Great Plains, providing evidence for the biotic resistance hypothesis. In Chapter 2, I investigate the interaction between drought and herbivory by the biological control agent, *Diorhabda carinulata*. Under which environmental conditions or geographical locations can biological control be maximized? Finally, in Chapter 3, I speculate

whether hybrid tamarisk individuals or populations differ in plant performance and herbivore defense traits. Since the biological control agent coevolved with one parent species, *T. ramosissima*, I hypothesized that some hybrids may be more or less susceptible to attack by this herbivore. Success of biological control may hinge upon the level of species introgression, and if hybridization occurs predictably across the landscape, managers can exploit this information for tamarisk control. My research not only attempts to improve control strategies, but also addresses fundamental questions in plant-insect ecology and evolution.

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DEDICATION

To my grandmother, the matriarch of my family and one of the smartest, kindest persons I know. Thank you for your love and support.

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CHAPTER 1: NATIVE STEM-BORING BEETLES (COLEOPTERA: BOSTRICHIDAE) EXTENSIVELY AND FREQUENTLY FEED ON INVASIVE *TAMARIX*

SUMMARY

Native stem-boring beetles (*Amphicerus bicaudatus*) were found feeding on stems of invasive tamarisks (*Tamarix*) at Bonny State Park, Yuma County, in eastern Colorado. At 11 sites in Colorado, Nebraska, and Kansas, I examined tamarisks and the most common co-occurring native tree, plains cottonwoods (*Populus deltoides*), for the occurrence of this beetle. Evidence of feeding and development of insects was detected at nine of the sites and in 112 of 579 tamarisks. Among sites, *A. bicaudatus* occurred in nearly 20% of tamarisks. Only 13 of 480 plains cottonwoods showed signs of activity by *A. bicaudatus*. During tamarisk stem dissections, nearly 90% of adult beetles were in live versus dead stems.

INTRODUCTION

Invasive exotic species are the second leading cause for extinction and decline in biodiversity worldwide, surpassed only by habitat destruction (Wilcove et al. 1998). Negative effects of invasive species include altering structure and function of ecosystems (Vitousek et al. 1987, Mack et al. 2000), decreasing abundance of endemic species (Wilcove et al. 1998, Mack et al. 2000), and accruing annual costs over $100 billion in lost goods or control measures in the United States (Pimentel et al. 2005). Therefore, determining ecological traits that affect invasion success is critical for predicting, preventing, and mitigating damages caused by biological invasions.

The biotic-resistance hypothesis is one attempt at explaining the outcome of invasions by exotic species. The hypothesis states that invaders encounter evolutionarily novel native enemies and competitors, which in turn limit their establishment or spread (Elton 1958,

Hokkanen and Pimentel 1989, Maron and Vila 2001, Keane and Crawley 2002). In the past decade, evidence for this hypothesis has been building (Agrawal and Kotanen 2003, Parker and Hay 2005, Parker et al. 2006, Jensen et al. 2007, Prider et al. 2009). Given that invaders are not adapted to certain pressures exerted upon them by native consumers, one possibility is that generalist herbivores in the introduced range could have potential negative effects on colonizing plant species.

In 2008, numerous tamarisk trees (*Tamarix* sp.) were observed to be infested with the native stem-boring beetle, *Amphicerus bicaudatus* (Coleoptera: Bostrichidae) at Bonny State Park, Yuma County, in eastern Colorado. Few native herbivores feed on tamarisks, and none are reported to inflict enough damage to significantly reduce performance or fitness of plants (Hopkins and Carruth 1954, Liesner 1971, Stevens 1985, Wiesenborn 2005). *Tamarix* is a complex of species that is among the most notorious invaders in the western United States (Gaskin and Schaal 2002). In the mid-19th century, at least eight tamarisk species were introduced for controlling erosion and for landscaping (Baum 1967). Four of these species have become highly invasive and hybridize readily, making identification to species problematic (Gaskin and Schaal 2002, 2003). Following the initial observation of *A. bicaudatus* in tamarisks, I conducted a field survey to determine if this insect was rare in tamarisks, a local phenomenon, or whether it occurred across a wider geographical range. Furthermore, I examined whether *A. bicaudatus* equally preferred the locally abundant and native *Populus deltoides* subsp. *monilifera* (plains cottonwood) as a host. If the insect frequently was attacking the invasive plant, its presence could lend support to the biotic-resistance hypothesis.

The biology of *A. bicaudatus* has received little attention except where it has been reported as a pest in grape vineyards and apple trees. According to Read (2008) and Hesler et al.

(2007), adult beetles (length of body, 10 mm) emerge April-May from overwintering sites in branches. At this time, adults presumably mate and make new entries to feed on dead wood. Eggs (1 mm long) are laid singly in crevices in bark on dying or dead branches during mid-May through June. Larvae (1-8 mm long) hatch soon after oviposition and burrow into the pith of stems where they complete development. By August, pupae appear in the galleries. New adults emerge from these now-dead stems and fly in search of live branches where they feed and overwinter. Feeding in live stems reduces productivity of hosts by 30-50% (Hesler et al. 2007) and eventually kills the infested stem (Beiriger et al. 1988). Reported hosts include native mesquites (*Prosopis*), exotic pears (*Pyrus*) and tamarisks, and exotic and native plants belonging to the genera *Carya* (hickory and pecan), *Fraxinus* (ash), *Malus* (apple), *Prunus* (apricot, cherry, peach, and plum), and *Vitis* (grape) (Popenoe 1888, Fisher 1950, Beiriger et al. 1988, Read 2008). The range of *A. bicaudatus* is restricted to the Great Plains and the eastern United States (Fisher 1950, Hesler et al. 2007).

Although *A. bicaudatus* was detected in tamarisks in the USA during the late 19th century (Popenoe 1888), no further literature has been published on this insect-plant interaction. Beyond examining its rate of occurrence in tamarisks, the second objective was to describe characteristic aspects of its infestation in tamarisks.

METHODS

To measure extent of infestations of *A. bicaudatus* in tamarisk, eleven sites from four watersheds in Colorado, Nebraska, and Kansas were selected for surveys (Table 1.1). All sites had invasions of tamarisks (Robinson 1965). Sites were visited during August-November 2008. I haphazardly chose ≥30 but ≤60 individual plains cottonwoods and tamarisks at each site for surveys. Incidence rates of the insect were compared in plains cottonwoods and tamarisks

because these species were the only locally abundant species of trees at all sites. *Salix exigua* and *S. amygdaloides* (coyote willow and peachleaf willow, respectively) occurred at some sites but never in high numbers. Each tree was examined for 2 min in search of evidence of activity by beetles (e.g., entrance holes and frass). If evidence was detected, the tree was recorded as being a host of *A. bicaudatus*. If no evidence was detected, *A. bicaudatus* was recorded as being absent on the tree. For those trees where *A. bicaudatus* was present, I recorded whether activity was from a previous year and subsequently abandoned, or whether it was current activity. It was also noted whether activity was in dead or live stems of the host by examining the stem for living or leafy tissue above the entrance holes. Samples of activity by beetles were collected by clipping stems below galleries. These stems were brought to the lab to confirm presence of *A. bicaudatus*, to collect individuals, and to measure basic characteristics of galleries. For each stem that was collected, length of galleries, number and diameters of entrance holes per gallery, and number and life stages of *A. bicaudatus* encountered were noted. The diameters of infested stems and whether they were living or dead at time of collection were also recorded. At one of the field sites (John Martin Reservoir) herbivory by *Plectridera scalator* (Coleoptera: Cerambycidae) was detected in a few cottonwood trees. Damage by this insect was easily distinguished by the larger and flatter shape of gallery construction compared to galleries caused by *A. bicaudataus*. *P. scalator* was never found in tamarisk nor was it found in plains cottonwood from other field sites.

A 100-m transect was established at the initial discovery site at Bonny State Park to get a second measurement of percentage occurrence and, thus, verify the haphazard surveying method and to obtain characteristics on those tamarisks that were selected as hosts versus those that were not. The nearest tamarisk every two meters along the transect was selected for observation.

Along with recording presence or absence of *A. bicaudatus*, I also took measurements of number of main stems arising at the base of the plant and diameters of three main stems at their base (later averaged to provide a general measure of size). Diameters were measured using dial calipers accurate to 0.1 mm (SPI Supplies, West Chester, Pennsylvania). The software program, SAS version 9.2, was used for statistical analyses (SAS Institute, Inc., Cary, North Carolina). Means are reported ± SE.

RESULTS

The native stem-boring beetle, *A. bicaudatus,* was present at nine of the 11 sites and at all watersheds surveyed. The insect was 8.6 times more likely to be in tamarisks than in plains cottonwoods (Wald $X^2 = 51.4$, df = 1, $P < 0.01$). Overall, the beetle was detected on 112 of 579 (19.3%) tamarisks (range at sites, 0-85.7%; Table 1.1). Conversely, *A. bicaudatus* was detected in only 13 instances out of 480 (2.7%) plains cottonwood trees sampled (range at sites, 0-11.7%). One-half of characteristic galleries of *A. bicaudatus* in tamarisks were vacant and presumed to be from previous feeding by adults or larvae. Of current activity of *A. bicaudatus*, 10% of that detected during haphazard surveys occurred in live stems. In plains cottonwoods, all galleries were short (<1 cm) and no beetles were found.

Among the 61 galleries in tamarisks that were dissected, average length of gallery was 8.5 cm (±1.1), average number of entrance holes/gallery was 1.5 (±0.1), and average diameter of entrance holes was 3.6 mm (±0.1). There were 1.4 (±0.2) adults per infested stem, and 89% of live beetles were in live stems of tamarisks. Average diameter of infested stems was 10.4 mm (±0.9) with a range of 3.5-18.0 mm. I collected a total of 75 adults and 4 larvae of *A. bicaudatus*. Voucher specimens were cataloged at the C. P. Gillette Museum of Arthropod Diversity

(Colorado State University, Fort Collins) and Montana Entomological Collection (Montana State University, Bozeman).

Of the 50 tamarisks along the 100-m transect, eleven had evidence of infestation by *A. bicaudatus*. There was no difference in number of basal stems between infested and uninfested trees ($t = 0.16$, $df = 48$, $P = 0.87$; mean number of basal stems/infested tree = 5.1 ± 0.6, mean number of basal stems/uninfested tree = 5.3 ± 0.5). Infested trees tended to have basal stems with larger diameters (41.6 ± 4.2 mm) than uninfested stems (36.4 ± 2.2 mm). However, this trend was not statistically significant ($t = 1.11$, $df = 48$, $P = 0.27$).

DISCUSSION

This study demonstrates that the native stem-boring beetle, *A. bicaudatus* attacks invasive tamarisks over an extensive area and at a relatively high frequency. Conversely, populations of native plains cottonwoods are not used frequently by *A. bicaudatus*. Until the current study, the relatively high densities of this insect in tamarisks and characteristics of its damage had not been recorded. At Bonny State Park, the initial site of discovery, haphazard surveys and a 100-m transect were used to find *A. bicaudatus* in 28 and 22% of tamarisks by each method, respectively. At other sites, occurrence of *A. bicaudatus* in tamarisks reached 85%, a considerable amount of infestation given the invasive nature of the plant and the rare reports of native herbivores feeding on this species (Hopkins and Carruth 1954, Liesner 1971, Stevens 1985, Wiesenborn 2005).

The native plains cottonwood has not been reported as a host of *A. bicaudatus*. The surveys of 480 plains cottonwoods revealed no beetles and short galleries, supporting the idea that this native insect occasionally samples plains cottonwoods but never feeds extensively or develops on this species. Because tamarisks from populations on the Great Plains seem to be

susceptible to attack by *A*. *bicaudatus*, the exotic tree might lack necessary defensive mechanisms against this generalist herbivore. However, four Old World species of bostrichid beetles attack various species of tamarisks in Israel (Halperin and Damoiseau 1980). Nonetheless, pressure exerted by *A*. *bicaudatus* on tamarisks at sites in the Great Plains may aid native species in their competitive struggle against the exotic plant (i.e., the biotic-resistance hypothesis). This phenomenon also may help explain why tamarisks rarely reach population densities as high as those west of the Continental Divide where *A*. *bicaudatus* does not exist.

A high percentage of living, adult *A*. *bicaudatus* was encountered in live stems of tamarisks during dissections, and all abandoned galleries (indicating previous activity by *A*. *bicaudatus*) were in dead stems. Both of these observations suggest that *A*. *bicaudatus* might be killing stems of tamarisks. However, it would be premature to assume that activity of this insect is causing mortality of stems. Multi-year experiments are currently being conducted in order to evaluate whether *A*. *bicaudatus* is capable of killing live stems of tamarisks, what effects it may have on fecundity of tamarisks, and if its preferred host is live versus dead stems of tamarisks. In addition, other experiments that determine functional and numerical response of the insect to tamarisks are needed to validate whether this particular scenario contributes to the biotic-resistance hypothesis (Maron and Vila 2001).

In their expanded range, all invaders encounter evolutionarily novel enemies and competitors that they are not adapted to deter (Maron and Vila 2001, Mitchell et al. 2006, Parker and Gilbert 2007). Although biotic resistance by native predators can stop the spread of invasive species (Nunez et al. 2008), evidence that biotic resistance can completely repel or prevent establishment of exotic species is in short supply (Maron and Vila 2001, Levine et al. 2004, Parker et al. 2006, Fausch 2008). Overall, little is known about demographic effects of native

herbivores on populations of invasive plants (Maron and Vila 2001, Liu and Stiling 2006). The dynamics between native *A. bicaudatus* and invasive tamarisks may help shed light on the biotic-resistance hypothesis and, perhaps, lead to a better ecological understanding of what regulates species invasions.

Table 1.1. Summary of activity of the stem-boring beetle (*Amphicerus bicaudatus*) in tamarisks (*Tamarix* sp.) and plains cottonwoods (*Populus deltoides* subsp. *monilifera*) at 11 sites surveyed during 2008.

Watershed	Site	Number of plains cottonwoods with activity by *A. bicaudatus*/number of plains cottonwoods surveyed (%)	Number of tamarisks with activity by *A. bicaudatus*/number of tamarisks surveyed (%)
Arkansas River	Florence, Colorado	0/30 (0)	0/30 (0)
	John Martin Reservoir, Colorado	7/60 (11.7)	11/60 (18.3)
	Purgatorie River, Colorado	1/30 (3.0)	8/60 (13.3)
	Rocky Ford, Colorado	0/60 (0)	8/60 (13.3)
	Thurston Reservoir, Colorado	3/60 (5.0)	27/60 (45.0)
Republican River	Bonny State Park, Colorado	0/30 (0)	17/60 (28.3)
	Swanson Reservoir, Nebraska	0/60 (0)	1/60 (1.7)
Solomon River	Kirwin Reservoir, Kansas	0/30 (0)	30/35 (85.7)
	Webster Reservoir, Kansas	2/60 (3.3)	3/60 (5.0)
South Platte River	Atwood, Colorado	0/60 (0)	7/60 (11.7)
	Ford Bridge, Colorado	---	0/34 (0)
Total		13/480 (2.7)	112/579 (19.3)
Average percentage among sites ±SE		2.3 ±1.2	20.2 ±7.7

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CHAPTER 2: VARIATION IN RESPONSE TO DROUGHT STRESS AND HERBIVORY BY A BIOLOGICAL CONTROL AGENT AMONG POPULATIONS OF THE INVASIVE SHRUB TAMARISK (*TAMARIX* SP.)

SUMMARY

Tolerance to defoliation by herbivores can be affected by environmental stress, but how herbivory and resource availability interact is difficult to predict. Conventional wisdom states that plants with high resource availability should be able to compensate for losses due to herbivory compared to plants that live in stressful environments (Compensatory Continuum hypothesis, CCH). The Limited Resource Model (LRM) aims to improve predictive power by taking into account particular resources that are limiting plant fitness and resources that are affected by herbivores. I designed an experiment to test the CCH and LRM using the invasive shrub, tamarisk (*Tamarix* sp.) and a specialized herbivore. I also investigated whether herbivore tolerance differed among tamarisk populations because these populations are known to differ in traits such as root allocation and leaf phenology. Overall, drought-stressed plants had higher tolerance to defoliation in terms of plant height and canopy vigor compared to well-watered plants, a result that is consistent with the LRM but not the CCH. Lateral stem length also showed patterns consistent with the LRM but tolerance was not significantly different between the two watering regimes. Although the three-way interaction between tamarisk population, herbivory, and drought stress was not significant for any of the plant performance variables, there were significant two-way interactions. Tamarisk populations originating from southern U.S. latitudes were more drought-sensitive than those from northern populations. Additionally, the southernmost population in the study had significantly less tolerance to defoliation than the other tamarisk populations. Last, southern populations grew larger than northern populations.

Together, these population differences represent an inherited latitudinal cline in tamarisk life history traits in the introduced range, which may be driven by differences in interspecific hybridization between two *Tamarix* species. The genetic differences in life history traits may also help explain why some populations of tamarisk are difficult to control using biological control agents. Experimental tests of plant defense theory can be useful in applications such as biological control.

INTRODUCTION

Damage from herbivory can have a profound impact on plant fitness. Generally, herbivory acts negatively on plants by decreasing vegetative and reproductive biomass and directly or indirectly reducing fruit production and seed set (Belsky 1986, Crawley 1989, Herms and Mattson 1992, Hawkes and Sullivan 2001). However, in other cases the effects of herbivory may be minimal or even beneficial to plants because some species are capable of replacing lost tissues (McNaughton 1983, Maschinski and Whitham 1989) or in fact grow larger after being released from apical dominance (Gao et al. 2008). The ability of plants to compensate for lost tissues is considered a defense strategy against herbivory and is referred to as tolerance (Strauss and Agrawal 1999, Tiffin and Inouye 2000). Despite our knowledge that compensation for herbivory directly translates into fitness benefits, the environmental factors most important in determining plant tolerance to herbivory are unclear (Hawkes and Sullivan 2001, Wise and Abrahamson 2005, 2007).

The availability of basic resources required by plants to grow and reproduce (e.g. light, CO_2, water) are thought to play an integral role in tolerance of herbivory. For example, some have argued that a plant's ability to compensate for herbivory will increase with increasing resource availability (Coley et al. 1985, Maschinski and Whitham 1989). This idea was later

coined the Compensatory Continuum Hypothesis (CCH) by Hawkes and Sullivan (2001). In short, the CCH predicts that a plant's tolerance to herbivory should be greater in high-resource, low-competition environments where losses to herbivores will be more easily replaced compared to low-resource, high-competition areas. Despite its logical appeal, recent reviews have shown less than outstanding support for the CCH. In two meta-analyses, the CCH accurately predicted results of plant performance only 31% and 53% of the time, respectively (Hawkes and Sullivan 2001, Wise and Abrahamson 2007). Clearly, the relationship between resources and tolerance is more complex than the simplistic CCH portrays.

The Limited Resource Model (LRM) was constructed to address the shortcomings of the CCH (Wise and Abrahamson 2005). The LRM takes into consideration how different forms of herbivory influence resource uptake, as well as which resources are likely to be limiting for plant fitness. Consider a scenario where plants are growing in a dry environment (i.e. water limited) and are simultaneously attacked by root herbivores. The LRM predicts that both the environment and this type of herbivory will act synergistically to reduce plant fitness. Alternatively, if the herbivore were an aboveground leaf feeder, uptake of an alternative resource (e.g. light or CO_2) would be affected by this particular form of plant consumption (e.g. reduction in photosynthetic area). In this case, the LRM predicts that fitness losses due to the folivore under drought conditions will be minimal because plant fitness is already severely limited by water availability as opposed to light or CO_2. Well-watered plants, on the other hand, would in fact be limited by some other resource, such as light or CO_2 (Wise and Abrahamson 2005). Therefore, plants in a well-watered environment would have less tolerance to leaf herbivory than plants grown in a water-limited environment because these plants are light-limited, and defoliators primarily affect this resource. The LRM provides a testable framework and takes into

consideration more complex interactions between herbivory and plant resources (Wise and Abrahamson 2005).

There has been mixed support for the LRM's ability to predict how tolerance to different herbivores will shift with resource availability. On one hand, the LRM accurately predicted 39 of 41 cases (95%) in a meta-analysis of published studies by Wise and Abrahamson (2007). Moreover, three recent direct tests of the LRM have found support for the model (Ramirez and Verdugo 2009, Sun et al. 2010, Bagchi and Ritchie 2011). A similar number of studies, however, have provided little to no support of the LRM (Gao et al. 2008, Gonzales et al. 2008, Atala and Gianoli 2009, Gianoli et al. 2009). Thus, more tests of the LRM are required before any definitive conclusion can be made regarding its predictive power.

Knowledge of plant vulnerability under particular stressful conditions has direct applications in agriculture and resource management. For instance, classical biological control of weeds is a method of rejoining introduced weeds with specialized natural enemies from their native range in an attempt to achieve top-down population regulation (DeBach 1964, Wapshere et al. 1989, Coombs et al. 2004, van Klinken and Raghu 2006). Releasing biological control agents in areas that vary in resource availability for host plants may lead to different effects on target populations. By understanding the circumstances that lead to the greatest reduction in plant performance, biological control practitioners can reduce costs and maximize control efforts. In a broader sense, causes of plant invasion may be due in part to interactions between enemy release and high resource environments (Blumenthal 2005, 2006).

In this study, I designed an experiment which simultaneously tested the CCH and LRM using the invasive weed, tamarisk (*Tamarix* sp.), and a specialized leaf-feeding biological control agent. Several species of tamarisk were introduced to the western U.S. in the mid nineteenth

century for stream bank stabilization and horticultural purposes (Baum 1967). At least four species in the genus are capableof interspecific hybridization, and backcrossed hybrids of two parent species, *T. ramosissima* and *T. chinensis*, make up the bulk of the U.S. invasion (Gaskin and Schaal 2002, Gaskin and Kazmer 2009). By the end of the twentieth century, these tamarisk hybrids became the second most abundant woody riparian plant taxon in the interior western U.S. (Friedman et al. 2005). Tamarisk invasions alter fire and flooding regimes and negatively affect the abundance of native plants and animals (Di Tomaso 1998, Bailey et al. 2001). By the 1980s, a biological control program was initiated by the U.S. government to help control tamarisk populations, especially in remote regions of its introduced range (DeLoach et al. 2003). Since tamarisk is a riparian, facultative phreatophyte found in semi-arid habitats, I asked how drought stress influences degree of susceptibility to damage by the control agent.

Open field releases of the control agent, *Diorhabda carinulata* (Coleoptera: Chrysomelidae) were carried out at sites across the western United States starting in 2001 (DeLoach et al. 2003). Beetle populations have become established on tamarisk stands in western Colorado, Utah, Nevada, New Mexico, and northern Arizona (Milbrath and DeLoach 2006, Moran et al. 2009). Both larvae and adults feed on tamarisk foliage and are capable of defoliating entire trees (Lewis et al. 2003). Repeated defoliation by large aggregations of *D. carinulata* apparently leads to mortality of tamarisk shrubs in as little as three years (Hudgeons et al. 2007, Pattison et al. 2011). However, data from several tamarisk biocontrol monitoring sites reveals large variation in tamarisk control by *D. carinulata* herbivory (pers. obs.). Moreover, common garden studies and manipulative experiments indicate that tamarisk populations have diverged in terms of allocation to belowground storage tissues and leaf phenology (Sexton et al. 2002, Friedman et al. 2008, 2011). Population differences in these life

history traits could affect tolerance to defoliation and recovery following drought stress, and may explain differences of biological control efficacy in the field. Therefore, a secondary goal of the study was to investigate potential population differences in tolerance to herbivory and drought stress. Elucidating the interactions between drought stress and herbivory by this control agent may help guide more efficient control programs for this invasive plant.

METHODS

Propagating plants

Cuttings were harvested in June of 2008 from mature plants being grown outdoors in a common garden at the Colorado State Forest Service in Fort Collins, CO. These plants were part of a larger study on the evolution of cold-hardiness and leaf phenology of wild U.S. populations along a latitudinal gradient (Friedman et al. 2008, 2011). Tamarisk shrubs in this common garden were three years old and 0.5-3 m tall at the onset of the present study. I collected replicate cuttings (20-30 cm length, 4-9 mm diameter) from 20 trees from each of six populations for a total of 120 individuals (Table 2.1). The cuttings were planted in 25-cm tree pots (Size D40, Stuewe & Sons, Tangent, Oregon) with 100% perlite. Cuttings were then placed on a mist bench with a misting regime of 15 sec duration every 3 min for 9 h/day over 4 weeks. Survivors were transplanted into 41-cm tree pots (Size TP616, Stuewe & Sons, Tangent, Oregon) in 100% sand. A slow release fertilizer (Osmocote, 14-14-14 NPK) was added at the time of transplanting. The plants were kept in a greenhouse (25^{o}C day/18^{o}C night temperature, 60-80% relative humidity, 14 h light/10 h dark) throughout the winter of 2008-2009. In May of 2009, I moved the plants to an outdoor garden on the Colorado State University campus.

Experimental treatments

I randomly selected five plants within each population to be subjected to each level of a 2-way factorial treatment design of drought stress and herbivory. This provided a balanced design of latitude (6 levels), water (2 levels) and herbivory (2 levels). On 5 June 2009, I took initial measurements of plant fitness (see below) prior to experimental manipulations. Also on this day, I started the drought stress treatment by controlling the amount of water being delivered to each pot using a drip irrigation system. Control plants received full watering to field capacity where soil media was saturated and allowed to drain roughly three times per week. Drought-stressed plants were watered as needed to maintain ~60% field capacity calculated gravimetrically. Average watering interval for the drought treatment was about one time per week. A subset of potted plants was weighed weekly to ensure proper watering durations. The gravimetric water contents of the high and low water treatments correspond to a soil water potential (ψ_{soil}) of -0.01 kPa and -0.12 kPa, respectively.

For the herbivory treatment, one half of the plants (n=60) were randomly assigned to herbivory by two generations of *D. carinulata.* The other half of the plants served as a control (no herbivory) by spraying once with a 6% solution of Bt insecticide. A pilot study showed that this concentration was sufficient to kill 100% of *D. carinulata* adults and larvae (W. Cranshaw, pers. comm.). Plants in the herbivory treatment were sprayed with a similar amount of water without insecticide. A mixture of 25 first- and second-instar larvae was put on each plant assigned to the herbivory treatment while no larvae were placed on the control plants. The two generations of larvae were placed on the plants assigned to the herbivory treatment on 7 July and 28 July 2009, respectively. All larvae established in canopies where they were placed, and

canopies of herbivore and control plants never overlapped. I removed any residual larvae on 10 September 2009 at which point I resumed full watering of all plants.

The timing of drought and herbivory in my experiment were designed to reflect field conditions. In the western U.S., where *D. carinulata* populations are established, the insect completes two to three generations of development before reproductive diapause in mid-August, allowing tamarisk shrubs in the area time to recover from herbivory before senescence in October (Lewis et al. 2003, Bean et al. 2007). Moreover, fall monsoon rains are common in the semi-arid western United States, sometimes flooding riparian corridors where tamarisk grows. Therefore after roughly one month of no herbivory and full watering of all experimental plants, I took the final fitness measurements on 1 Oct 2009. The entire experiment from induction of drought to the last fitness measurement was 17 weeks.

Plant measurements

Only three of the 120 plants in the experiment produced flowers so no direct fitness measurements could be recorded. Instead, I measured the size and vigor of plants at the beginning and end of the experiment. Plant performance is a suitable proxy for fitness (Mauricio et al. 1993, Huhta et al. 2003). The length of the tallest stem arising from the root crown was recorded as plant height. I measured lateral growth by randomly marking three side branches with metal twist ties before treatments were initiated. Their lengths were measured as the distances from their branching points to their tips. The three lengths were summed for each plant to provide a single measurement. Hereafter, this measurement is referred to as stem length. Finally, I recorded subjective score of plant vigor for each individual. Plant vigor was based on a scoring system of yellow or dead foliage of each plant where I defined 75-100% yellow/dead foliage = 1, 50-75% = 2, 25-50% = 3, and 0-25% = 4. A higher vigor score reflects a healthier

plant canopy. Tolerance was determined by comparing average absolute plant performance between the two herbivory treatments.

Statistical analyses

Analysis of covariance (ANCOVA) was carried out using JMP v.9.0.2 (SAS Institute, Cary, NC). The main effects of water, herbivory, and population were modeled as fixed factors. I considered population a fixed effect because I was curious as to whether plant performance varied among plant populations, as was the case of cold-hardiness and leaf phenology (Friedman et al. 2008, 2011). To that end, I coded plant population as a categorical variable based on latitude of origin (Table 2.1). For all ANCOVAs, the three-way interaction among the main effects was included but was not significant for any response variable. Therefore, I omitted it from the models and ran each analysis again. Initial plant size or condition was included as a covariate in the ANCOVA models. This statistical design tests for the effects of drought stress, herbivory and population while adjusting for initial size or condition of plant subjects in the experiment.

RESULTS

Survivorship was excellent over the 17-week experiment. Only one plant from the low water, herbivore treatment died. The single dead plant was retained in all statistical analysis of plant performance. By the end of the herbivore treatment, larvae were still present on 65% of plants to which they were applied. Only one larva was found on a control plant during the experiment, and it was promptly removed. Although defoliation from herbivory was not directly measured, damage to plant canopies from feeding larvae was estimated to be 25-75%.

Plant height

Drought stress and herbivory both reduced plant height ($F = 46.7$, $df = 1,100$, $P < 0.0001$; $F = 24.7$, $df = 1,100$, $P < 0.0001$, respectively). They also interacted in their effects ($F = 5.2$, $df = 1,100$, $P = 0.03$), such that plants under drought stress remained relatively short statured even in the absence of herbivory relative to those plants subjected to herbivory. Thus, plants with higher resource availability were less tolerant of the defoliating herbivores than plants with low resource availability (Fig. 2.1A).

Plants from different populations varied in their response to drought stress in terms of plant height (population*water: $F = 3.1$, $df = 5,100$, $P = 0.01$). Plants from southern latitudes grown under high water availability were taller than northern plants grown under the same conditions; when grown under drought conditions this difference disappeared (Fig. 2.2A). Similarly, plants from different populations responded differently to herbivory (population*herbivory: $F = 3.2$, $df = 5,100$, $P = 0.01$). In this case, the significance was attributed to differences in only the southernmost population, Buffalo Lake, TX (Fig. 2.3A). Control plants from this population were taller (LS mean ± SE: 67.0 ± 4.5 cm) than plants in the same population in the herbivore treatment (LS mean ± SE: 30.6 ± 4.6 cm). On its own, the main effect of population was significant for plant height ($F = 2.6$, $df = 5,100$, $P = 0.03$) with plants from the southernmost population growing taller than the two northern most populations (Table 2.2). Finally, the covariate of initial plant height significantly influenced final plant height ($F = 50.1$, $df = 1,50$, $P < 0.0001$).

Stem length

Data on stem length from five plants were lost due to missing stem markers, and thus, these plants were excluded from the analysis of stem length. Drought stress significantly

reduced stem lengths ($F = 33.3$, $df = 1,95$, $P < 0.0001$; Fig. 2.1B), but stem lengths were not altered by herbivory ($F = 0.7$, $df = 1,95$, $P = 0.39$). There were no significant interactions among the main terms (water*herbivory: $F = 2.3$, $df = 1,95$, $P = 0.14$; population*water: $F = 1.1$, $df = 5,95$, $P = 0.37$; population*herbivory: $F = 1.6$, $df = 5,95$, $P = 0.16$). However, there were significant pairwise differences between drought-stressed and control plants and between herbivore treatments across populations when analyzed using Student's t test (Fig. 2.2B, Fig. 2.3B). Plants from southern sites had longer stem lengths than those from northern sites (Table 2.2).

Plant vigor

For scores of plant vigor, the interaction between water and herbivory was significant ($F = 29.8$, $df = 1,100$, $P < 0.0001$). Plants in the high water treatment always had higher vigor than plants in the drought-stress treatment, but the effects of herbivores on vigor was more pronounced in the high water treatment than the drought-stress treatment (Fig. 2.1C). The main effects of water ($F = 233$, $df = 1,100$, $P < 0.0001$) and herbivory ($F = 34.5$, $df = 1,100$, $P < 0.0001$) were also highly significant on their own. The effect tests showed no significant interactions between population and water ($F = 0.4$, $df = 5,100$, $P = 0.85$) or between population and herbivory ($F = 0.2$, $df = 5,100$, $P = 0.96$) on plant vigor. The main effect of population also was not significant for plant vigor ($F = 0.3$, $df = 5,100$, $P = 0.93$). When using Student's t-tests for pair-wise comparisons, I did not detect any population differences for drought stress or tolerance to herbivory (Table 2.2). The covariate of initial vigor did not have an effect on final plant vigor ($F = 2.2$, $df = 1,100$, $P = 0.14$).

DISCUSSION

I designed an experiment to test two popular models of how resource availability affects an invasive plant's tolerance to herbivory. Both the CCH and LRM predict higher fitness of plants when they are grown in high versus low resources in the absence of herbivory, which proved to be the case in the current study. Performances, as indicated by plant height, stem length and plant vigor, increased to higher values under well-watered conditions compared to drought-stress conditions. The predictions of the CCH and LRM differ, however, when pressure from aboveground leaf herbivory is considered along with water availability. The CCH predicts lower tolerance of leaf herbivory under low water availability while the LRM predicts lower tolerance under high water availability. In my study with the biological control agent, *D. carinulata*, tamarisk shrubs grown under well-watered conditions had lower tolerance compared to shrubs grown under drought-stressed conditions for two of the response variables: plant height and vigor. Herbivores did not significantly affect the third plant measurement, stem length, although there was a trend for lower tolerance at higher water availability similar to that for the other two measures of plant performance. As such, the predictions of the LRM are supported by the tamarisk-*D. carinulata* system.

Traditional theoretical models investigating plant response to herbivory in stressful environments have recently come under scrutiny because of their low predictive power (Hawkes and Sullivan 2001, Wise and Abrahamson 2007). The Limited Resource Model developed in 2005 by Wise and Abrahamson attempts to improve upon models such as the Compensatory Continuum hypothesis by considering the types of herbivores and particular limiting resources involved. Since the publication of the model, a handful of studies have directly tested the LRM using defoliation of aboveground tissues while simultaneously manipulating water delivery to

plants. Contrary to the results in this study, an annual vine (*Ipomoea purpurea*) showed less tolerance to defoliation under drought conditions in terms of shoot biomass (Atala and Gianoli 2009). Similarly, relative growth rates of the perennial grass, *Leymus chinensis*, actually increased with increasing amounts of defoliation but only under well-watered conditions (Gao et al. 2008). The perennial herb, *Convolvulus demissus*, has reduced tolerance to defoliation under drought conditions, both in terms of plant biomass and root-to-shoot ratios (Gianoli et al. 2009, Quezada and Gianoli 2010). In contrast, an equal number of studies show increased tolerance under drought conditions (Bagchi and Ritche 2011, Ramirez and Verdugo 2009, Sun et al. 2010). Particularly interesting is a comparison between two conspecifics: invasive *Alternaria philoxeroides* and native *A. sessilis*. The exotic plant has increased tolerance to defoliation while the native plant had decreased tolerance under drought conditions, thus providing a possible explanation as to why the introduced plant becomes invasive in stressful environments (Sun et al. 2010). Others have suggested that tradeoffs may exist between tolerance and other herbivore defense strategies such as, physical or chemical defenses, and these tradeoffs may differ depending on the type and severity of environmental stress (Stowe et al. 2000, Fornoni et al. 2004, Ramirez and Verdugo 2009). Given the importance of these alternative defense strategies (i.e. "resistance"), future investigations of the LRM should be designed to detect differences in this defense trait as well as tolerance (Mauricio 2000, Fornoni et al. 2004, Nunez-Farfan et al. 2007, Johnson 2011).

I found significant differences between populations in sensitivity to drought stress and herbivory for two of the three response variables. Plants from the four southernmost populations had greater reductions in plant height in response to drought than the two northern populations (Fig. 2.2A-B), and herbivores affected one particular population to a greater extent compared to

the other populations (Fig. 2.3A-B). It would be interesting to know whether this pattern will hold true upon further experimentation. Finally, when considering the effect of population on its own, southern plants grew taller and wider than plants from northern populations (Table 2.2). Since this study was conducted in a common garden environment, population differences in plant performance, drought sensitivity and tolerance to herbivory appear to suggest a genetic component of these traits (Colautti et al. 2009).

The population differences in tamarisk growth traits reflect a latitudinal cline in climatic conditions where the source plants were originally collected. If latitudinal clines persist when offspring of the plants are grown in a common environment, the differences in traits represent inherited genetic variation as opposed to phenotype plasticity (Maron et al. 2004, Colautti et al. 2009). Latitudinal clines in growth rate and reproduction are common among native plant species but only recently have they been discovered in invasive plants in their introduced ranges (Weber and Schmid 1998, Kollmann and Banuelos 2004, Maron et al. 2004, Friedman et al. 2008, Montague et al. 2008). Tamarisk is one such invasive plant that appears to have evolved a latitudinal cline, particularly in allocation to belowground tissues, cold hardiness, and leaf phenology (Sexton et al. 2002, Friedman et al. 2008, 2011). In the current study plants from southern populations grew larger than those from northern populations, perhaps because they allocate more resources to above vs. below-ground tissues than northern populations or because they retain their leaves longer into the fall than northern populations (Friedman et al. 2011, Williams et al. in prep.). These differences in morphology appear to reflect climatic conditions and lengths of growing season at latitudes where the source material was originally located. The mechanisms for this evolution are not fully known. Changes in genetic variation could have resulted from chance sampling events, hybridization, natural selection, or a complex interaction

among all of these factors (Lee 2002, Prentis et al. 2008). The evolution of increased size or tolerance to drought among tamarisk populations in this study appears to be adaptive, but experimental tests relating these traits to fitness benefits need to be conducted.

Latitudinal clines in drought tolerance, plant architecture, or other natural history traits could reflect levels of species introgression in the tamarisk invasion. The tamarisk invasion in the western U.S. has a high rate of hybridization where 83-87% of all plants have varying levels of introgression between *T. ramosissima* and *T. chinensis* (Gaskin and Kazmer 2009, Williams et al. in prep.). Of the two species involved in the hybrid swarm, *T. chinensis* parents and backcrossed-hybrids occur in the south (29-33 ^{o}N) while *T. ramosissima* parents and backcrossed hybrids occur in the north (44-48 ^{o}N). Hybrid tamarisks in the middle latitudes represent a 50/50 mixture of these two parent species. Varying levels of introgression between these two species could explain tolerance to herbivory or drought more so than latitude of population origin alone. For instance, *T. chinensis* is from subtropical eastern Asia while *T. ramosissima* originated from the desert-steppe of central Asia (Baum 1967). It is reasonable to think that *T. ramosissima* has evolved higher drought tolerance than that of *T. chinensis*, and these traits are now apparent in hybrids in the introduced range. Indeed, tamarisk from northern populations (i.e. high *T. ramosissima* introgression) had higher tolerance to drought than tamarisk from lower latitudes. Similarly, *T. ramosissima* genotypes, given their long evolutionary history with *D. carinulata*, could have adapted higher tolerance to defoliation than populations dominated by *T. chinensis* alleles. However, only one population in this study differed in tolerance to defoliation so care must be taken when interpreting population or genotypic differences. A more comprehensive test is required to elucidate the relative importance of tamarisk populations versus the genetic effect of hybridization on such plant traits as growth rate, tolerance to herbivory, and resistance.

If latitudinal or hybridization patterns exist, they can be exploited to the benefit of biological control programs.

One shortcoming of the present study is that I did not directly measure amount of herbivore damage on plants. Even though the same number of larvae were placed on each plant and the plants all appeared to respond with similar amounts of defoliation (circa 25-75%), variation in defoliation could be attributed to differences in resistance to herbivory. By not recording the actual amount of defoliation, it is hard to assess whether tolerance or resistance plays a greater role in this system (Wise and Abrahamson 2005). However, a similar outdoor garden experiment investigating resistance of introduced tamarisk plants from a range of latitudes did not show differences in resistance in terms of canopy damage (Williams et al., in prep). Therefore, it is reasonable to assume that damage levels were somewhat consistent across all plants in the experiment assigned to the herbivory treatment, and differences detected in plant performances do indeed reflect variation in tolerance. Still, it would be interesting to investigate whether other resistance measurements differed among hybrid tamarisk populations or by levels of species introgression. Along with differences in tolerance to herbivory, information on population or genotype differences in resistance could have direct applications.

The major weaknesses of LRM in general are its two principal assumptions: a focal resource is most limiting for plant fitness and that herbivory affects one resource more than any other (Wise and Abrahamson 2007). The first assumption is easy to detect in a manipulative experiment when herbivores are not present: plant performance must be significantly higher in high focal resource conditions versus low resource conditions. In my study, this first assumption was met as evidence of taller plants, longer stems, and higher leaf vigor under high water, no herbivory conditions. The second assumption of the LRM is harder to verify. Certainly, leaf

herbivory removes photosynthetic area and thus strongly affects the acquisition of light and CO_2. However, herbivory by *D. carinulata* has also been shown to decrease water use efficiency in tamarisk (Synder et al. 2010). Therefore, although the pattern revealed by these data largely support the LRM, more complex interactions involving water loss, light, and CO_2 may be contributing to this particular system. Some authors have concluded that the LRM is too broad and must be split into separate or revised hypotheses investigating the roles of resources on tolerance to herbivory (Gao et al. 2008, Banta et al. 2010).

My test of the LRM using an invasive shrub and a biological control agent is a novel approach on two levels. First, Wise and Abrahamson's (2007) meta-analysis of the LRM included only one woody perennial species out of the 41 species in the study. In the time since their review was published, only one additional study investigated the interaction of water availability and herbivory on a woody plant (*Populus* sp.) for which the authors found a similar result of lower tolerance under drought conditions (Ramirez and Verdugo 2009). Additionally, my study is the first of its kind to test population differences of an invasive plant in response to simultaneous defoliation by a biological control agent and drought stress. Although, there were no population differences in tolerance across water resource, the fact that some tamarisk populations demonstrated differences in drought or herbivore tolerance separately is potentially useful in an applied sense. Tamarisk populations in southern U.S. latitudes may be less tolerant of *D. carinulata* herbivory and appear more sensitive to drought stress; therefore, if limited funds are available managers could focus their attention in these locations. Moreover, the effects of biological control on tamarisk growth will be maximized in areas with high water availability. It would be useful to understand more concretely the tolerance differences and potential resistance

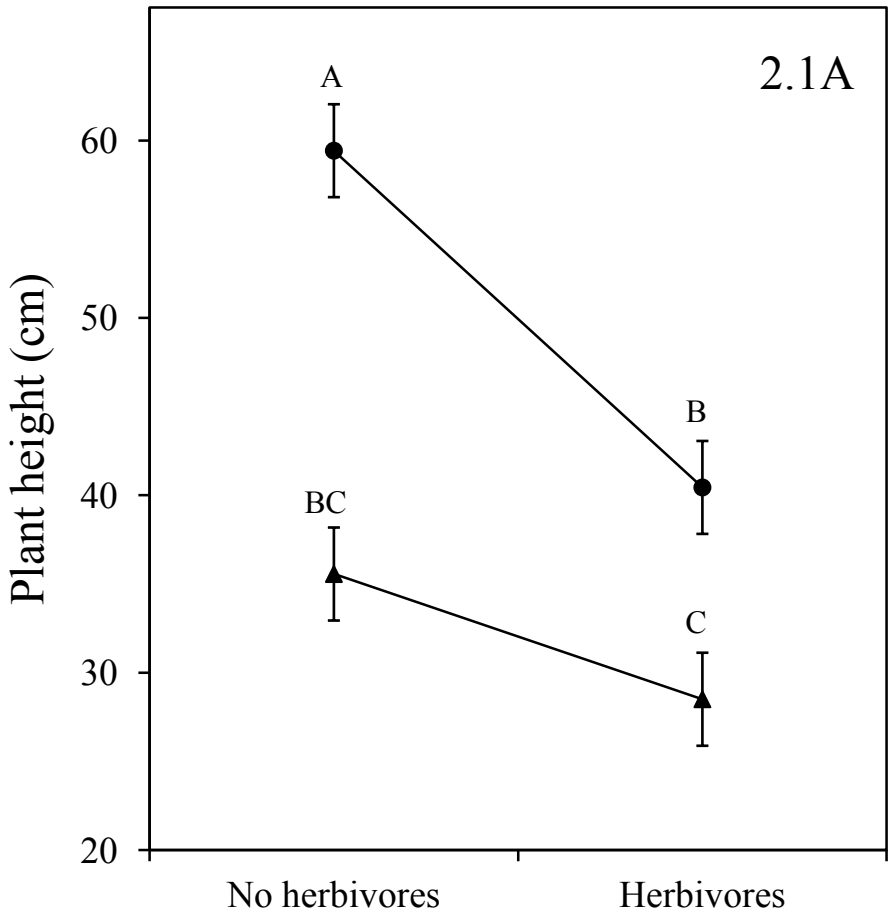
differences in tamarisk populations and whether these differences are related to species hybridization of this invasive plant.

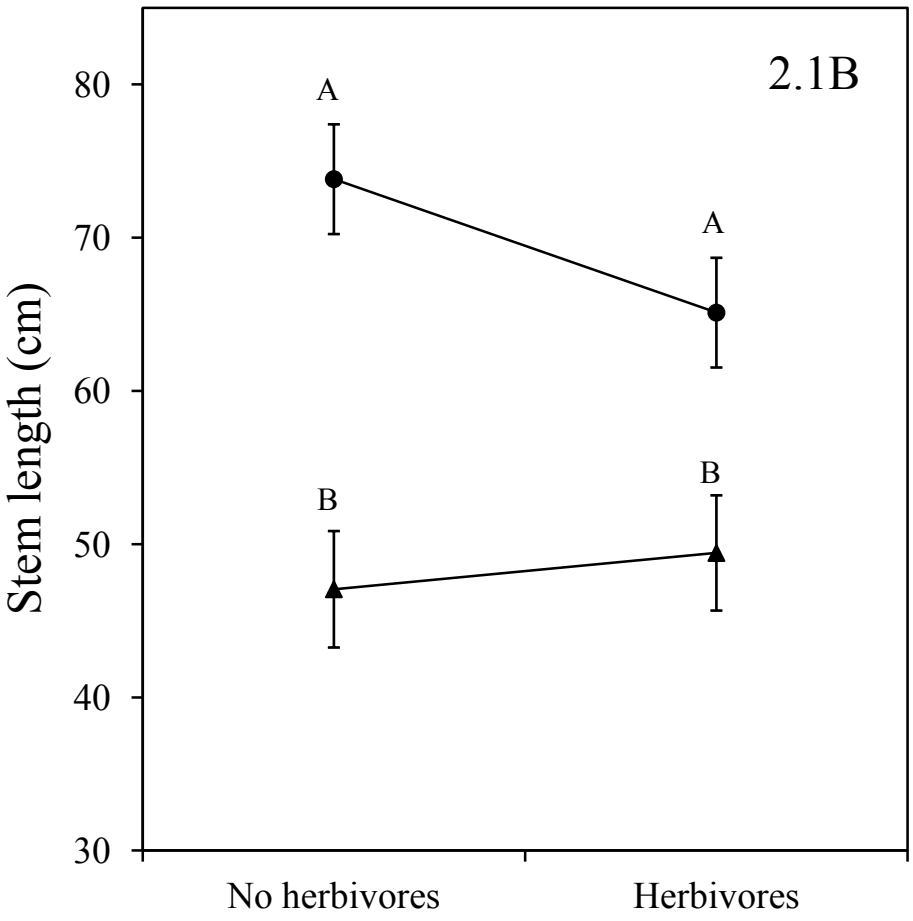
Table 2.1. The coordinates and elevations of the six sites where tamarisks in this study were originally collected. *=location and elevation of the common garden.

Population	Code	Latitude (^{o}N)	Longitude (^{o}W)	Elevation (m)
Buffalo Lake, TX	35	34.904	-102.118	1106
Cimarron River, OK	37	37.122	-101.892	1031
Lake McConaughy, NE	41	41.291	-101.933	999
Keyhole Reservoir, WY	44	44.368	-104.792	1251
Powder River, MT	45	45.427	-105.405	923
Fort Peck Reservoir, MT	48	47.604	-106.902	686
Fort Collins, CO*	-	40.573	-105.082	1529

Table 2.2. Population differences in the three plant performance measurements. LS means (±SE) for plant height, lateral stem length, and plant vigor. Different letters indicate significant differences among the populations using Student's t-tests. (α=0.05).

Population	Code	Plant height (cm)	Stem length (cm)	Plant vigor
Buffalo Lake, TX	35	48.8 (3.2) A	64.9 (4.4) A	2.59 (0.10) A
Cimarron River, OK	37	41.9 (3.5) AB	64.9 (4.6) AB	2.59 (0.10) A
Lake McConaughy, NE	41	41.6 (3.2) AB	58.5 (4.4) AB	2.54 (0.10) A
Keyhole Reservoir, WY	44	43.4 (3.2) AB	55.6 (4.5) AB	2.69 (0.10) A
Powder River, MT	45	34.7 (3.3) B	57.3 (4.9) AB	2.58 (0.10) A
Fort Peck Reservoir, MT	48	35.5 (3.2) B	52.0 (4.6) B	2.59 (0.10) A


2.1A
A
B
BC
C
60
50
40
30
20
Plant height (cm)
No herbivores
Herbivores


2.1B
Stem length (cm)
80
70
60
50
40
30
A
A
B
B
No herbivores
Herbivores

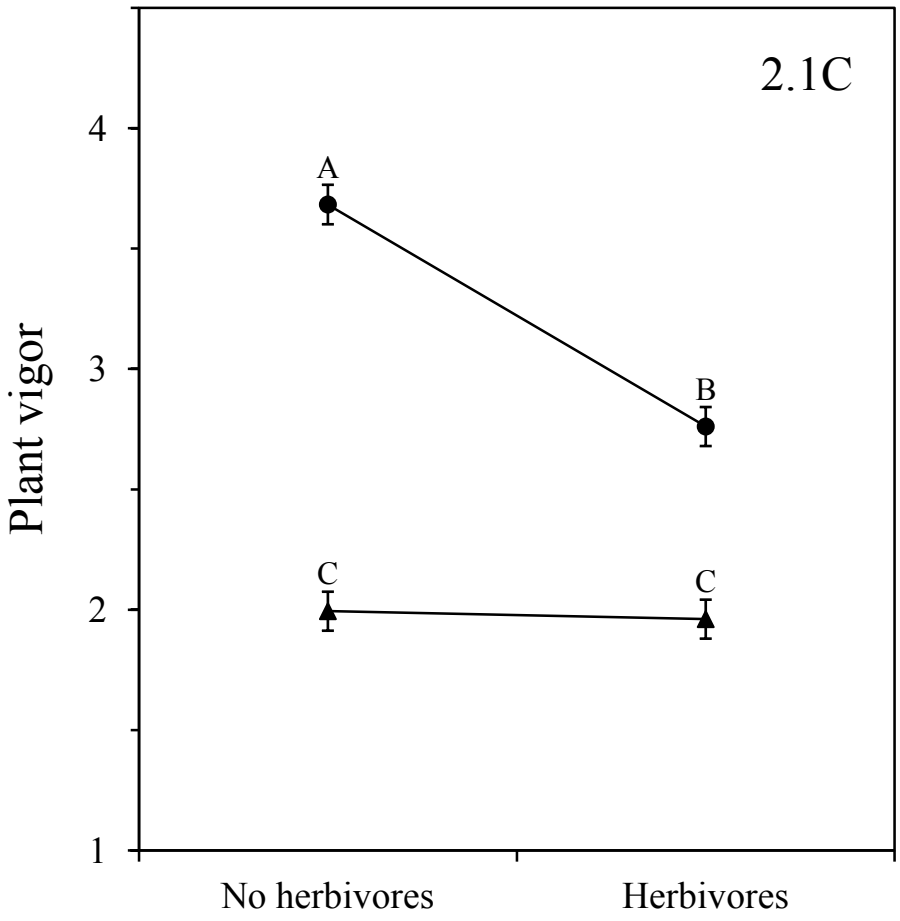
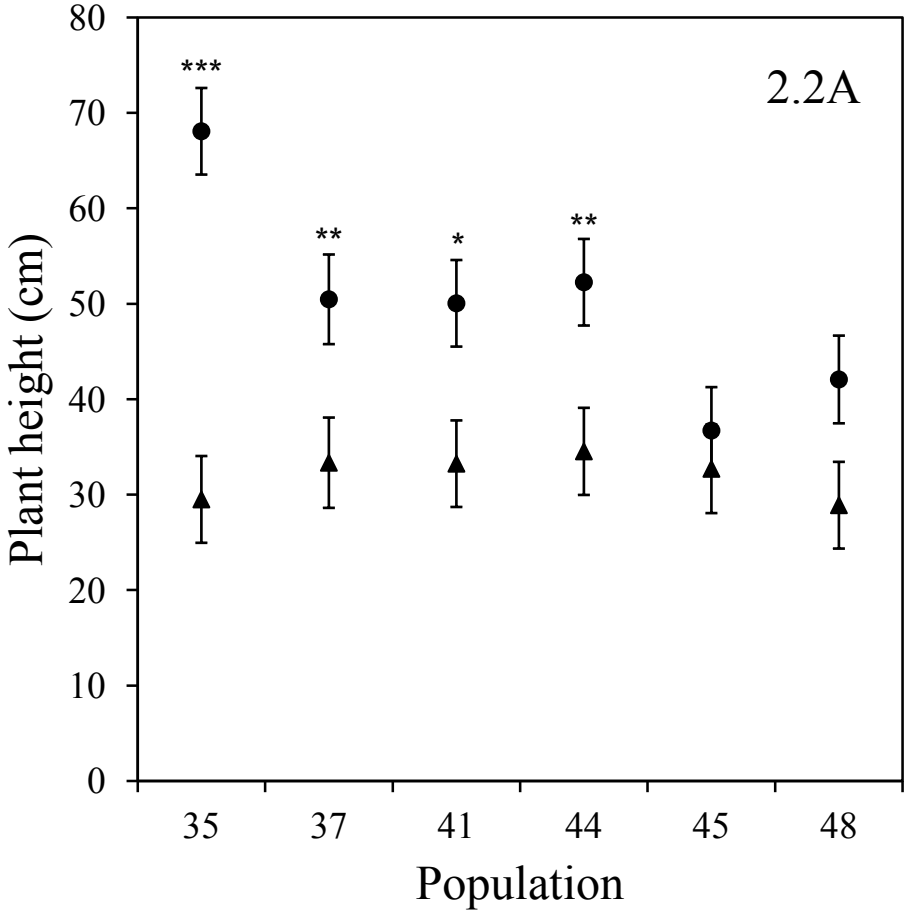


Figure 2.1. Plant responses to herbivory and high (circles) and low (triangles) water availability. LS means (±SE) of plant height (2.1A), lateral stem length (2.1B), and vigor (2.1C) following 13 weeks of reduced watering and 9 weeks of herbivory by *D. carinulata*. Different letters indicate significant differences among the means.


80
70
60
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40
30
20
10
0
Plant height (cm)
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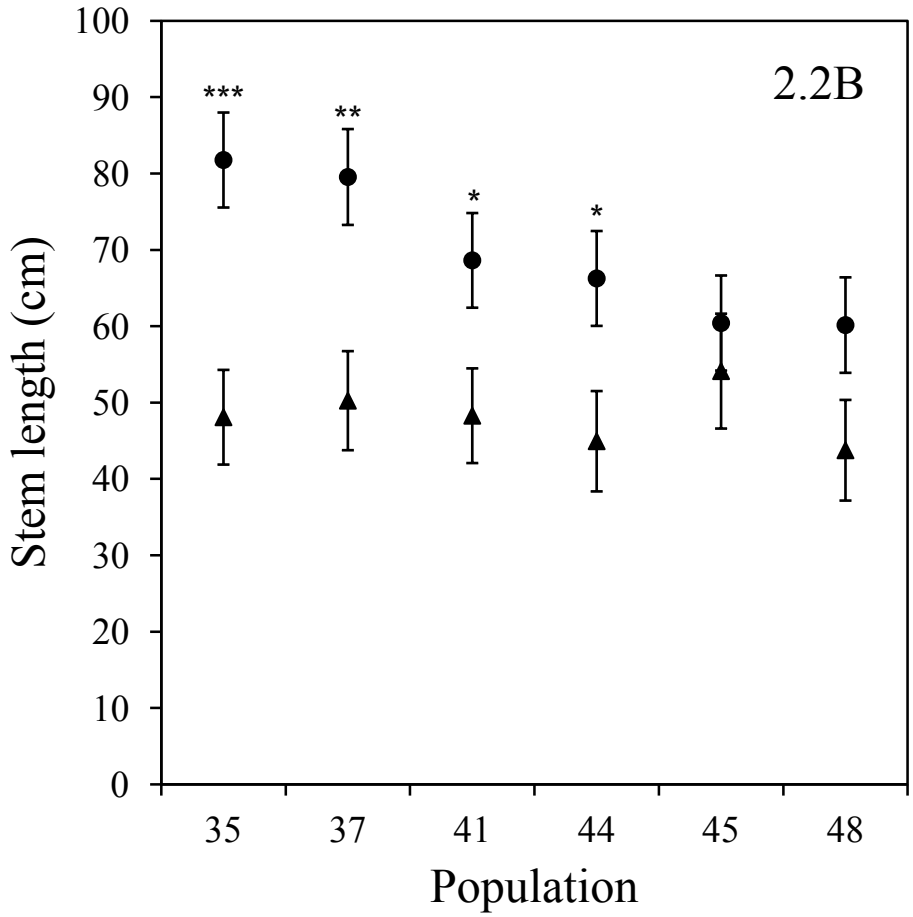
*
**
2.2A
35
37
41
44
45
48
Population


2.2B
Stem length (cm)
100
90
80
70
60
50
40
30
20
10
0
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*
*
35
37
41
44
45
48
Population

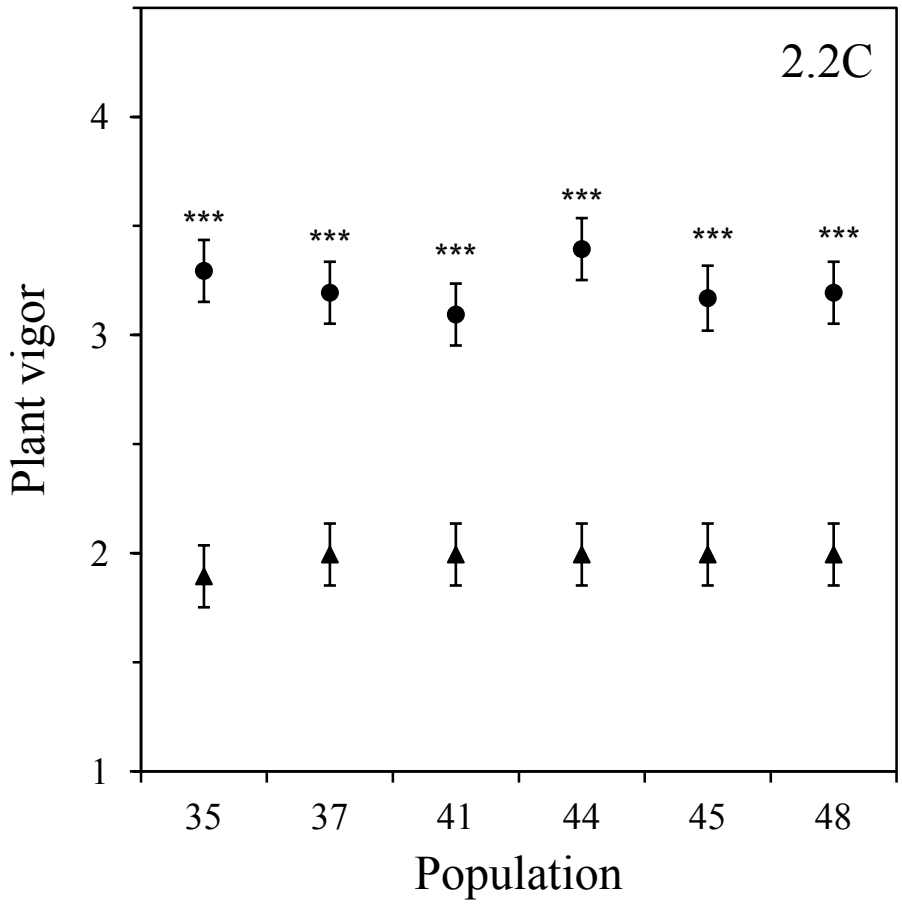
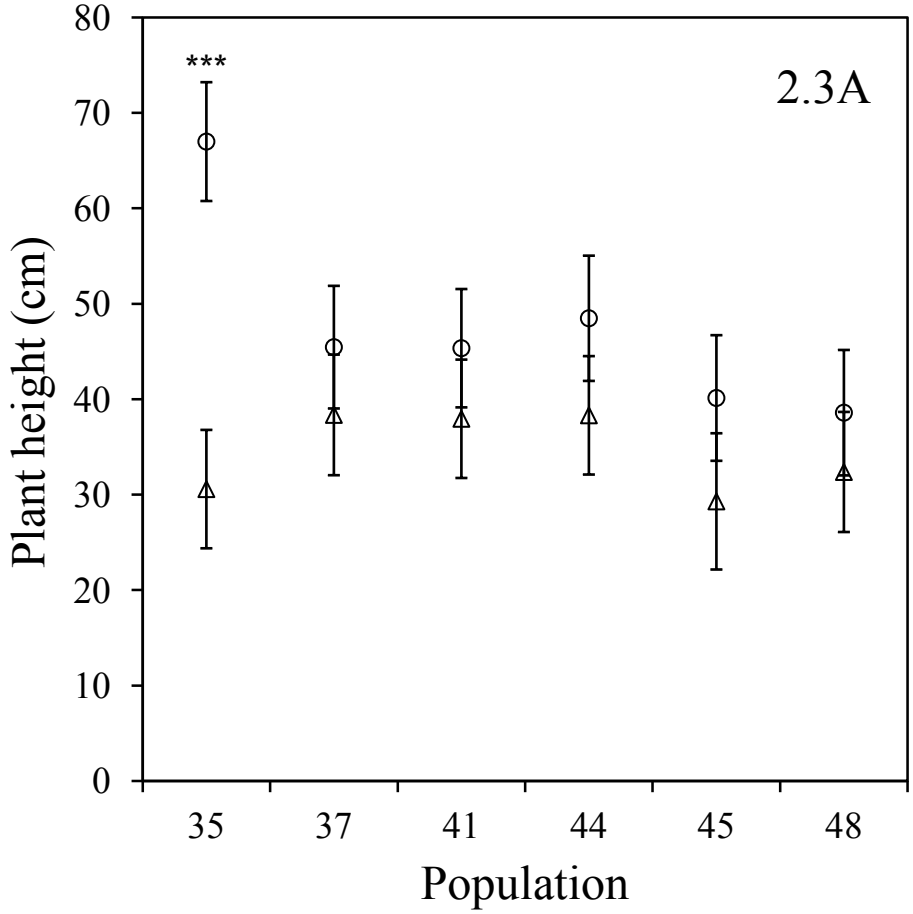
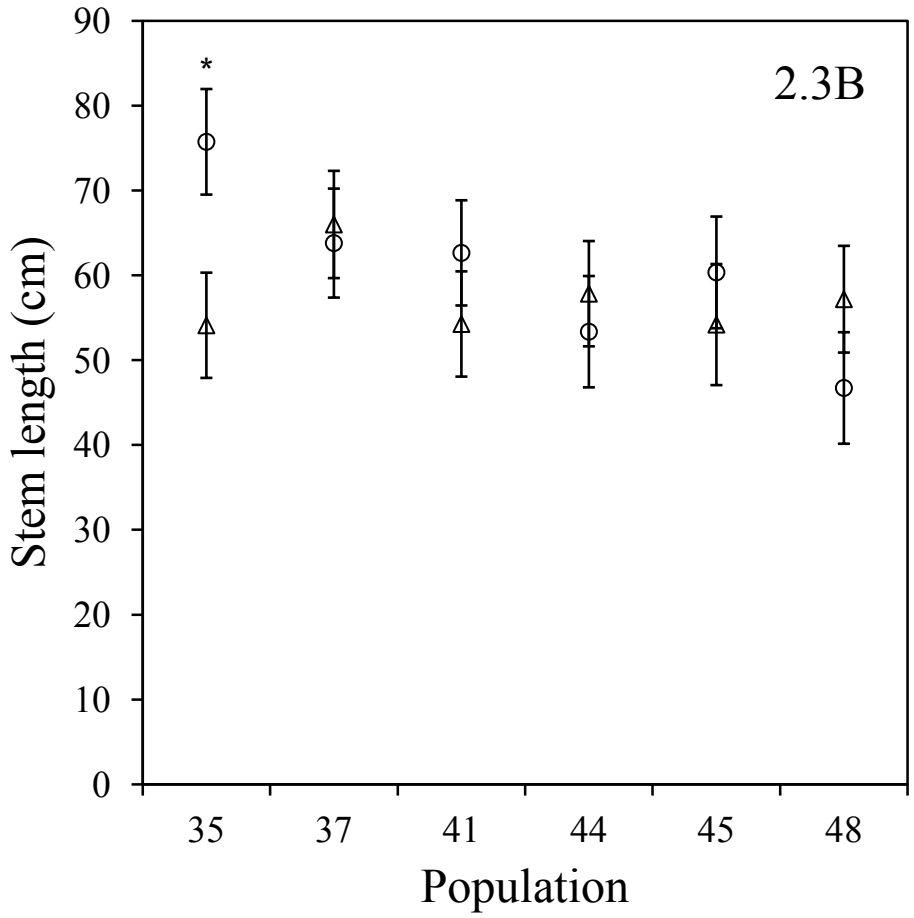


Figure 2.2. Population differences in plant response to drought stress. LS means (±SE) for plant height (2.2A), lateral stem length (2.2B), and plant vigor (2.2C). Asterisks refer to significant differences in means between high (circles) and low (triangles) water availability for a given population using Student's t-tests. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Populations are coded in terms of degrees latitude of their U.S. origin. See Table 2.1 for exact site locations.


80
70
60
50
40
30
20
10
0
***
2.3A
Plant height (cm)
35
37
41
44
45
48
Population


2.3B
*
Stem length (cm)
90
80
70
60
50
40
30
20
10
0
35
37
41
44
45
48
Population

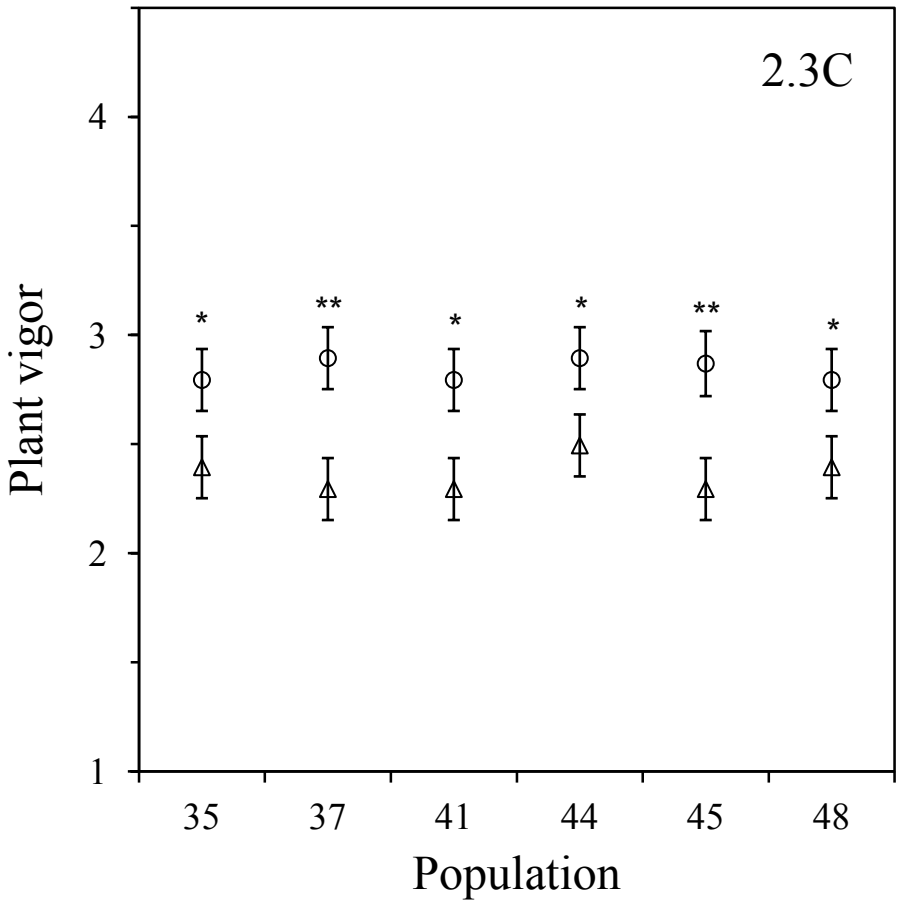


Figure 2.3. Population differences in plant response to herbivory. LS means (±SE) for plant height (2.3A), lateral stem length (2.3B), and plant vigor (2.3C). Asterisks refer to significant differences in means between control (open circles) and herbivore (open triangles) treatments for a given population using Student's t-tests. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Populations are coded in terms of degrees latitude of their U.S. origin. See Table 2.1 for exact site locations.

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CHAPTER 3: HYBRIDIZATION OF AN INVASIVE SHRUB AFFECTS TOLERANCE AND RESISTANCE TO DEFOLIATION BY A BIOLOGICAL CONTROL AGENT

SUMMARY

Evolution is an important process in determining the successful invasion of exotic plant species in their new introduced ranges. Adaptations to climatic conditions have been documented in several invasive plants using molecular data and common garden experiments. Natural selection and interspecific hybridization can both contribute to 'invasiveness'. How evolution of exotic plants via adaptation or hybridization affects interactions with specialized insect herbivores is unclear. A genotypic shift in plant traits, such as root allocation or chemical constituents, could affect outcomes of classical biological control. I used a model-selection approach to investigate the relative roles of evolved latitudinal clines and hybridization on plant performance and herbivore defense traits of the invasive shrub tamarisk (*Tamarix* sp.). Plants from 14 populations representing 32-48 ^{o}N latitude were grown in a common garden and subjected to defoliation experiments. I used AFLPs and Bayesian Cluster analysis to assign levels of species introgression for each plant in the garden. There was a strong correlation between *T. ramosissima* introgression and latitude ($r = 0.86$), making it difficult to disentangle the effects of hybridization from latitude of population origin. Moreover, AIC model selection indicated that latitude and introgression explained nearly the same amount of variation in plant growth rate and biomass. However, introgression explained slightly more variation in root:shoot ratios and herbivore defense than latitude. As the percentage of *T. ramosissima* introgression increased, root:shoot ratios and tolerance to defoliation increased, suggesting that plants with high levels of *T. ramosissima* alleles are better able to cope with defoliation compared to plants with high levels of *T. chinensis* alleles. Conversely, a bioassay with plant material revealed that

high *T. ramosissima* introgression corresponded to low herbivore resistance and high larval performance. To definitively tease apart the effects of hybridization and latitude in this system would require quantitative trait loci mapping and breeding experiments involving parent species. Nonetheless, the differences in tolerance and resistance among tamarisk hybrids underpin the importance plant hybridization to interactions with herbivores and may explain why some biological control releases are more successful than others.

INTRODUCTION

Rapid evolution is an important process contributing to the success of invasive plant species in their introduced ranges (for reviews see Lee 2002, Bossdorf et al. 2005, Keller and Taylor 2008, Prentis et al. 2008). Some introduced plant species have evolved faster growth, higher fecundity, or altered allocation of herbivore defenses compared to populations in the native ranges (Blossey and Notzold 1995, Joshi and Vrieling 2005, Hull-Sanders et al. 2007). Moreover, evolution is invoked to explain why populations of several exotic plant species persist at low numbers for decades before undergoing massive growth and spread (e.g. termed 'evolution of invasiveness', Ellstrand and Schierenbeck 2000). Evolutionary mechanisms, such as founder events, intra- and interspecific hybridization, and adaptation, have all resulted in significant evolutionary change in the introduced ranges (Lee 2002, Bossdorf et al. 2005, Prentis et al. 2008). Not only do invasive plants species provide a unique opportunity to study evolution in contemporary time, it is also imperative to understand the evolutionary dynamics that influence invasion success since these species are related to declines in biodiversity, drastic changes in ecosystem function, and costs of over $120 billion annually in losses and control measures in the U.S. alone (Wilcove et al. 1998, Mack et al. 2000, Pimental et al. 2005).

Hybridization is an important evolutionary mechanism that has allowed introduced plant populations to overcome low genetic diversity associated with founder events and is considered a principal attribute to population growth and spread (Lee 2002). Although hybridization produces mostly maladaptive genotypes, it can also result in novel genotypes which may be better suited to their environment than either parent species (Lee 2002, Donovan et al. 2010). Hybridization may also transfer beneficial genes for traits such as cold hardiness or resistance to fungal diseases and herbivores (Snow et al. 1999, Milne and Abbott 2000, Abbott et al. 2003, Whitney et al. 2006, Rieseberg et al. 2007). Selection can act on these novel hybrids, spreading beneficial alleles rapidly throughout populations of invasive plants (Keller and Taylor 2008). Indeed, hybridization has been implicated in numerous invasive plant species (for reviews, see Ellstrand and Schierenbeck 2000 and Schierenbeck and Ellstrand 2009), with several hybrid taxa evolving larger sizes or more fecundity than either parent species, or outcompeting and replacing parent species altogether (Campbell et al. 2006, Whitney et al. 2006, Ridley and Ellstrand 2010). Invasive plant hybrids indirectly threaten native communities and pose difficulty to land managers who are responsible for their control (Vila et al. 2000, Blair et al. 2008).

An effective tool for managing invasive plants is classical biological control, where specialized natural enemies are imported and released for the purposes of providing top-down effects on their hosts (DeBach 1964, Wapshere et al. 1989, van Klinken and Raghu 2006). How biological control is affected by invasive plant hybridization and other evolutionary mechanisms remains largely unanswered (Muller-Scharer et al. 2004). In native systems, herbivores can distinguish among hybrid genotypes (Fritz et al. 1998, McGuire and Johnson 2006), and hybrid plants can have more or less resistance to attack compared with their parent species (Whitham 1989, Fritz et al. 1999, Krebs et al. 2011). In invasive plant systems, hybridization may also play

a role in frequency of herbivore attack, especially when hybrids are compared as a group to their parent species (Blair et al. 2008, Krebs et al. 2011, Cuda et al. 2012). However, little is known about how susceptibility to herbivory varies across levels of species introgression in invasive plants. This information could be useful in cases where invasions are comprised almost entirely of hybrid genotypes (Williams et al. 2007, Gaskin and Kazmer 2009). Clearly, more research is needed to determine the extent to which invasive plant hybridization affects efficacy of classical biological control.

In addition to hybridization, another mechanism of evolution important to invasive plants is adaptation to abiotic stress (Bossdorf et al. 2005, Ridley and Ellstrand 2010). Introduced plants are exposed to novel climatic and edaphic conditions in the new range on which selection can act. One way this selection is evident is through the evolution of latitudinal clines. When grown in a common environment, populations of several exotic plant species exhibit inherited genetic differences in size, phenology, and cold hardiness reflecting the climate from the latitude where they were collected (Weber and Schmid 1998, Kollman and Bañuelos 2004, Maron et al. 2004, Leger and Rice 2007, Friedman et al. 2008, Montague et al. 2008, Monty and Mahy 2009, Keller et al. 2009). While latitudinal clines of native plant species have been well documented for quite some time (Turreson 1930, Clausen et al. 1940), similar patterns are also seen for non-native plants, some of which have evolutionary histories as little as 100 years in their new ranges (Ridley and Ellstrand 2010, Hodgins and Rieseberg 2011). It is not apparent whether evolution of strategies to cope with abiotic stress may interact with biological control. For instance, northern populations of *Tamarix* sp. may have evolved to allocate more resources to underground tissues due to winter dieback (Friedman et al. 2011). In turn, these same populations may have more tolerance to aboveground herbivory (i.e. more roots provide more

carbohydrate reserves for leaf flush following defoliation). Evolution of life history traits to cope with abiotic stress could influence biological control.

Here I investigate latitudinal clines and hybridization of the perennial shrub/tree tamarisk (i.e. saltcedar, *Tamarix* sp., family Tamaricaceae). Several species of tamarisk were introduced to the U.S. in the nineteenth century to stabilize stream banks and to serve as ornamental plants (Robinson 1965). By the mid-1900s at least four species were considered serious pests in the arid west (Gaskin and Schaal 2002, Gaskin et al. 2012). Currently, tamarisk occupies over 500,000 ha (Zavaleta 2000) and is the second most dominant woody riparian species in the western United States (Friedman et al. 2005). Negative consequences of tamarisk invasion include alteration of ecosystem functioning and decreases in biodiversity (Zavaleta 2000, Shafroth et al. 2005). In 2001, after a decade of research, the biological control agent, *Diorhabda carinulata* Brullé (Coleoptera: Chyrsomelidae) was released at field sites across western North America. In some locations, populations of this insect are well established and control of tamarisk is being achieved, while at other sites, the control agent has failed to establish despite repeated attempts (DeLoach et al. 2003, 2009). Some authors have speculated as to whether genetic makeup of plant hosts may play a role in these instances (Gaskin and Schaal 2002, Gaskin and Kazmer 2009). Indeed, field populations of *D. carinulata* that once preferred their co-evolved host, *T. ramosissima* over the sympatric *T. parviflora* have now adapted to equally prefer *T. parviflora* (Dalin et al. 2009, Thomas et al. 2010). Could preference for specific *Tamarix* genotypes be driving the success or failure of biological control?

Many of the introduced tamarisk species are capable of interbreeding to produce viable hybrids, but the hybrid offspring of two parent species, *T. ramosissima* and *T. chinensis*, make up the bulk of the invasion in the western United States (Friedman et al. 2008, Gaskin and Kazmer

2009). Based on diagnostic markers and AFLP data, Gaskin and Kazmer (2009) estimated 83-87% of genotypes collected across several sites in western North America were indeed hybrids. Additionally, out of 110 genotypes sampled only 14 individuals were classified as parent species. Interestingly, despite repeated attempts, no hybrid genotypes have been found in the native range (Gaskin and Schaal 2002, Gaskin and Kazmer 2009). Therefore, hybridization appears to be a major part of the tamarisk invasion in the U.S., but it is unknown how hybridization influences basic plant traits such as growth rate, morphology, and defense against herbivory (Gaskin and Kazmer 2009). In a set of experiments investigating cold hardiness and leaf phenology of tamarisk populations, Friedman et al. (2008, 2011) demonstrated inherited latitudinal variation in these traits. Southern populations had significantly increased susceptibility to cold temperatures and later onset of fall leaf senescence compared to northern populations when plants were grown in a common environment. The authors concluded that this latitudinal cline is most likely due to hybridization and natural selection, especially since there doesn't appear to be obstacles to gene flow in North America. There is no indication to what degree this latitudinal variation is attributed to levels of species introgression or whether variation in these traits influences interactions with biological control (e.g. tolerance and regrowth following defoliation).

My objectives for the current study were threefold: First, I aimed to quantify the relationship between *Tamarix* species introgression and latitude of population origin. Two previous studies have indicated that the parent species *T. ramosissima* and its backcrossed hybrids are common in northern latitudes while the parent species *T. chinensis* and its backcrossed hybrids are common in southern latitudes (Friedman et al. 2008, Gaskin and Kazmer 2009), but a definitive relationship is unknown. Second, I wanted to determine whether latitude of population origin or levels of species introgression could better explain interactions

with the specialized herbivore, *D. carinulata* and other basic plant growth traits such as response to defoliation, growth rate, and biomass allocation. The final goal of the study was to quantify the strengths of the relationships, if any, between latitude or hybridization and these plant traits. To test these ideas, I subjected clones of plant genotypes representing several populations to different defoliation treatments and measured plant response in growth. Overall, answers to these questions can potentially improve biological control in this system where evolution for at least some plant traits (i.e. cold hardiness, leaf phenology) has occurred.

METHODS

Plant material

Plant genotypes used in this study were originally collected for experiments investigating latitudinal gradients of cold-hardiness and leaf phenology of wild U.S. tamarisk (Friedman et al. 2008, 2011). For their experiments, Friedman and his colleagues collected stem cuttings from healthy shrubs growing in natural populations (~25 genotypes per population, 14 populations). The cuttings were planted in an outdoor common garden in Fort Collins, Colorado, where observational studies on survivorship and leaf phenology as well as tests of cold hardiness were conducted using harvested stem tissue. When the plants in the garden were three years old (0.5-3.0 m height), I haphazardly chose a subset of ten genotypes per population for my own common garden experiment (Table 3.1). The process of propagation using two successions of growing plants from cuttings should have negated any possible maternal effects.

I propagated six clones for each of the ten selected genotypes per population. Cuttings were harvested by clipping sections of woody stems 25 cm long and 5-8 mm diameter from side branches. I recorded stem diameter and used this measurement as initial plant size later in the statistical analyses. The cuttings were dipped in a 10x rooting hormone solution (1% indole-3-

butyric acid + 0.5% 1-Naphthaleneacetic acid, Dip N' Grow, Inc. Clackamas, Oregon) before being planted individually in 25-cm deep pots (Size D40, Stuewe & Sons, Tangent, Oregon) with 100% perlite. Cuttings were then placed on a mist bench with a misting regime of 15 sec duration every three min for nine h/day over four weeks. Survivors were transplanted into 41-cm treepots (Size TP616, Stuewe & Sons, Tangent, Oregon) with a mixture of 80:20:1 of potting soil, sand, and 14-14-14 NPK slow-release fertilizer. These plants were kept in the greenhouse under constant temperature and light until it was suitable for them to be relocated to an outdoor common garden on the campus of Colorado State University, Fort Collins, Colorado.

Outdoor experiment

Of the plants that survived propagation, three clones of each of 43 genotypes representing 10 populations were used in the outdoor experiment (Table 3.1). Each clone was randomly assigned to one of nine experimental blocks with no genotype occurring more than once per block. The blocks consisted of 100 cm W x 100 cm L x 30 cm H wood box frames. Blocks, and thus the plant clones contained within, were randomly assigned to one of three defoliation treatment groups. First, on 16 July 2010, I introduced 150 adult *D. carinulata* into each of three blocks assigned to the herbivore defoliation treatment. The beetles were collected from an established field population near Palisade, Colorado, and were starved 24 hours before their release. To contain them within the blocks, I placed cages constructed from lightweight nylon landscaping cloth and plastic tubing over the box frames. I removed these cages after 14 days when adults reached the end of their life cycle and larvae hadn't yet hatched from egg masses (Lewis et al. 2003). Once emerged, larvae were free to move and feed among overlapping plant canopies within each block while canopies from neighboring blocks were kept isolated. Larvae, which are not highly mobile, were never recorded outside of the blocks to which they were

assigned. Blocks of the other defoliation treatments (chemical and control) were caged in the same manner and timing as those in the herbivore treatment.

For plants in the three blocks assigned to chemical defoliation treatment, the non-systemic, defoliating herbicide, carfentrazone-ethyl (Aim™, FMC Corp. Philadelphia, PA) was applied with a four-nozzle boom and a backpack sprayer using two passes at a volume of 187 L per hectare, which is consistent with field application of this herbicide (S. Nissen, pers. comm.). The chemical defoliant was applied to the assigned plants well outside of the garden. The timing of the chemical application purposefully coincided with the height of beetle defoliation (13 August 2010). The remaining three blocks were assigned to the control treatment, and plants contained in these blocks did not receive any prescribed defoliation. This design allowed all genotypes in the experiment to have one clone in each of the three defoliation treatments and took into consideration environmental heterogeneity by splitting the treatments into blocks.

From June to October 2010, measurements of damage, plant performance, and fitness were recorded (see below). During this period, plants were watered every third day and supplemented with nutrients monthly using 20-20-20 NPK fertilizer. From October 2010 to April 2011, the wood boxes were filled with mulch to prevent roots from freezing. Water was supplemented as needed during this dormant stage. In April 2011, the plants were uncovered, and the watering and fertilizing regime continued until the end of the experiment in June 2011 when all plants were harvested.

Quantifying damage, plant performance, and tolerance

I used percentage of plant canopy damaged as a measure of defoliation. On 3 September 2010, I took two digital photographs of each plant against a black background. The photographs were analyzed using ImageJ (v1.44o, U.S. National Institute of Health, http://imagej.nih.gov/ij/).

The whole canopy area was determined by setting the hue threshold from 21 – 102. Next, the green canopy area was measured by setting the hue threshold from 47 – 102. I averaged the whole- and green-canopy areas for pairs of pictures for each plant. By comparing the green canopy to the whole canopy area, I was able to assess the percentage of canopy damaged for each plant. My technique of quantifying defoliation matched closely to a subjective score of percent plant damage (Pearson's correlation = 0.94, $p < 0.0001$).

Canopy volume was used as a proxy for plant fitness. In June 2010 and again in June 2011, plant height and the perpendicular canopy widths were used to calculate canopy volume. This measure of canopy volume was strongly correlated with canopy area calculated using digital images and ImageJ software ($r=0.79$, $p<0.0001$). For scores of tolerance to defoliation, I followed the methods of Strauss and Agrawal (1999) and quantified the slope of the relationship between damage and fitness. A positive slope indicates overcompensation for defoliation while a negative slope reveals under-compensation. When calculating tolerance, it is important to include only plants closely related to each other (Strauss and Agrawal 1999). Thus, I compiled 43 measures of tolerance, one for each genotype in the study with one clone of each genotype subjected to control, herbivore, and chemical defoliation.

At the end of the experiment (June 2011), I harvested and measured the biomass of the plants. The roots were carefully washed of potting soil and placed in separate paper bags for drying. Green foliage and woody aboveground growth (excluding the original cutting) were also put in separate bags. The samples were placed in an oven at 55^{o}C until they were dry (72 h). I used a digital balance to record the dry mass of green foliage, woody stems, coarse roots and fine roots. Coarse roots were defined as those ≥1mm diameter and fine roots as those <1mm in diameter.

Bioassay experiment

I developed a second experiment to investigate resistance of tamarisk genotypes to herbivory by *D. carinulata*. The remaining four populations that were not involved in the outdoor garden were used for this second study (Table 3.1). Five to eight genotypes per population and two to six clones per genotype survived the propagation technique described above. In August 2010, after these cuttings were transplanted and established in the outdoor garden for 10 weeks, I clipped ~30 g of fresh green foliage from each plant, placed the material in a paper sack, and dried for three days at 40^{o}C. Then, I powdered each sample using a coffee grinder, cleaning with 70% EtOH and drying between samples. Next, I added 5 g of dried plant material, 750 mg of agar, and 30 ml of H_2O to individual flat-bottomed glass test tubes. After mixing the solution, the test tubes were placed in a steam bath for 20 min and then cooled to room temperature. This method produced uniform artificial diets representing each individual clone grown in the garden. Two identical pellets from each diet were extracted from the tubes using a 5-mm punch. Pellets were placed on top of #1 Whatman filter paper inside of 50-mm plastic Petri dishes. One dish was randomly chosen as a control (no larvae) while the other received five, pre-weighed second instar *D. carinulata* larvae collected from field site in Palisade, Colorado. The larvae were starved for 24 h before the experiment began. The Petri dishes were placed in an environmental chamber for 48 h with a daily temperature (29 ^{o}C/21 ^{o}C) and light cycle (14 h L/8 h D). After 48 hours, larvae were removed and weighed. The pellets were then dried at 45^{o}C for 3 d. The dry masses of the 'herbivore' and 'control' pellets were then compared to obtain percent damage.

AFLPs

For DNA fingerprinting, I used identical plant clones that were not involved in the above experiments and followed the protocol of Gaskin and Kazmer (2009). In addition, I included all other tamarisk genotypes originally collected by Friedman et al. (2008). Genomic DNA was extracted from approximately 20 mg of silica-dried plant material using a modified CTAB method (Hillis et al. 1996). Ligation and restriction were carried out during the same step using T4 DNA ligase and buffer along with *Mse*I and *Eco*RI primers and adapters (New England Biolabs, Ipswich, MA). Preselective polymerase chain reaction (PCR) was performed over 20 cycles with the amplification primers *Mse*I + C and *Eco*RI + A. Next, the amplified product was diluted and added to the amplified primer pairs, *Mse*I + CTA/*Eco*RI + ACC and *Mse*I + CTA/*Eco*RI + ACT. The selective PCR was run for 10 cycles, and the *Eco*RI primer was dye-tagged with D4 (blue). The final selective product was loaded into an Applied Biosystems 3130 Genetic Analyzer (Foster City, CA). Fragments were first scored using software from the analyzer and then using GeneMapper v4.0 (Applied Biosystems) to visualize fragments for presence and length. Finally, the software program, Structure v2.3.3 (Pritchard et al. 2000, Falush et al. 2003, 2007; Hubisz et al. 2009), was used to calculate assignment scores and introgression levels in terms of one of the two parent species, *T. ramosissima*. The burn-in and Monte Carlo Markov Chain iterations were each set at 10,000 with assumed parent population size of two (*T. ramosissima* and *T. chinensis*). Most of the native *Tamarix* AFLP data used here was from Gaskin and Kazmer (2009). Plants were considered hybrids if assignment scores were <0.9 and posterior probability intervals did not reach 1.0 (Pritchard et al. 2000, Blair and Hufbauer 2010). I express species introgression in terms of percentage of one of the two parent

species, *T. ramosissima*, and hereafter refer to this measurement simply as 'introgression'. One can obtain *T. chinensis* introgression by simple subtraction (100-%*T. ramosissima* introgression).

After manual screening fragment output, the DNA analyzer registered 102 and 92 fragments between 50 and 500 bp for *Mse*I + CTA/*Eco*RI + ACC and *Mse*I + CTA/*Eco*RI + ACT, respectively. My AFLP analysis utilized the same laboratory, AFLP process and platform, as Gaskin and Kazmer (2009). Using this same method and equipment, Gaskin and Kazmer (2009) found an AFLP error rate of 1.2%, an equivalent of 2.33 miscalled fragments out of the 194 scored fragments per plant.

Statistical analysis

Because latitude of population origin and tamarisk species introgression are correlated (Gaskin and Kazmer 2009, this study), I used Akaike's information criterion (AIC) as a means to select the best-fit model between those investigating various plant responses to defoliation. AIC uses maximum log likelihood to estimate the relative difference between the fitted model in question and the unknown true model (Burnham and Anderson 2002). A lower AIC score reflects a better fit, and AIC scores between candidate models can be compared in this regard. A difference in AIC scores (Δ_i) <2 suggests substantial support for either model while $4<\Delta_i<7$ imply less support and Δ_i >10 indicates essentially no support for a candidate model (Burnham and Anderson 2002). I developed two model sets for the AIC model selection (Table 3.2). One model focused on latitude while the other on species introgression. Because the ratios of observations in my dataset to the number of parameters in the models were <40, I converted AIC to AICc scores, which take into account smaller sample sizes. I also calculated Akaike weights (w_i), the relative likelihood of a model given the data and competing models, and evidence ratios, the relative likelihood of model pairs, both of which are used as additional techniques for

determining the best fit model (Burnham and Anderson 2002). An evidence ratio >3 suggests there is relatively little evidence in favor of that particular model compared to the best-fit model (Burnham and Anderson 2002). I used PROC MIXED (SAS v9.3, SAS Institute, Cary, NC) with maximum likelihood estimation of error to calculate AICc scores.

I employed mixed-model analysis of covariance (ANCOVA) for investigation of defoliation damage and plant performance measurements using the statistical program JMP v9.0.2 (SAS Institute, Cary NC). Defoliation treatment and experimental block were considered fixed effects while plant subject was modeled as a random effect. Diameter of stem cuttings at the onset of plant propagation was used as a covariate to control for the effect of initial size. For Model 1 I included the continuous variable latitude and its interaction with defoliation treatment. For Model 2, I ran the same analysis but with species introgression and its interaction with defoliation treatment in lieu of latitude. In the bioassay experiment, variables in models investigating plant pellet resistance and larval performance included the random effect of plant subject and either latitude (Model 1) or introgression (Model 2). For tolerance, I used a weighted regression analysis to determine the strength of the relationship between species introgression and tolerance to defoliation. The weights were calculated as $1/SE^2$ where SE was the standard error for each of the 43 tolerance slopes (damage vs. fitness for each genotype). Finally, I used Student's t-tests (α=0.05) for post hoc comparisons among group means of treatment groups. Canopy area was log-transformed in order to meet model assumptions.

RESULTS

AFLPs

Tamarisk introgression was highly correlated with latitude of plant origin (Pearson's correlation = 0.86, $P < 0.0001$). The amount of tamarisk introgression increased with increasing

latitude in the western U.S. (%*T. ramosissima* introgression = 4.6*latitude - 123; ANOVA: F = 987, df = 1, 340, P < 0.0001; Fig. 3.1). Tamarisk from Colorado River (32.0 ^{o}N) had the lowest amount of *T. ramosissima* introgression (22.7 ± 1.7%) while tamarisk from Musselshell River (46.4 ^{o}N) had the highest amount of *T. ramosissima* introgression (92.9 ± 1.1%). Mean introgression across all latitudes was 59.3 ± 1.4%. Out of the 342 U.S. tamarisk shrubs included in the AFLP analysis, there were 37 individuals with assignment scores >0.9 for *T. ramosissima* and only four individuals with assignment scores >0.9 for *T. chinensis*. The individuals with high *T. ramosissima* introgression were collected from six locations: Poudre River (1 plant), Lake McConaughy (2 plants), Boysen Reservoir (5 plants), Keyhole Reservoir (3 plants), Musselshell River (20 plants), and Fort Peck Reservoir (6 plants). These sites represent all of the northernmost populations in the analysis (41.3-47.6 ^{o}N) with the exception of Powder River (45.4 ^{o}N), where all introgression levels were < 90% *T. ramosissima* (Table 3.1). Individuals with high *T. chinensis* introgression (i.e. >90%) were collected from four locations: Colorado River (2 plants), Lake Meredith (1 plant), and Cimarron River (1 plant). These sites represent three of the five southernmost tamarisk populations in the study (32.0, 35.5, and 37.1 ^{o}N, respectively; Table 3.1). The other two southern populations, Lake Alan Henry (33.1 ^{o}N) and Buffalo Lake (34.9 ^{o}N), had plants with introgression levels <90% *T. chinensis*.

Model selection

Since introgression was highly correlated with latitude of population origin (r=0.86), these two factors could not be placed in the same model investigating plant performance, thus justifying the use of AIC for model selection (Burnham and Anderson 2002). AIC scores showed that latitude (Model 1) and introgression (Model 2) similarly explained variation among response variables (Table 3.3). For damage and plant biomass, latitude and introgression were

equal in their ability to fit the observed data (Δ_i AIC < 2). For the other response variables in the outdoor garden experiment, canopy growth and root-to-shoot ratio, Δ_iAIC values were 4.0 and 4.6. These values indicate slight support for latitude and introgression, respectively. For the two response variables from the bioassay experiment, pellet resistance and larval performance, introgression explained more variation than latitude; however, the Δ_i AIC values were < 4 showing no clear support for either candidate model. The fact that no Δ_i was >10 shows that the effects of introgression and latitude of population origin cannot easily be disentangled (Burnham and Anderson 2002).

Plant performance traits

Because latitude and introgression explained similar amounts of variation in plant traits, I provide ANCOVA reports of all response variables for both candidate models (Appendix 1). Defoliation treatment had the same magnitude of effect and group means, regardless of whether the analysis included latitude or introgression. Initial plant size (cutting diameter) had a significant influence on all aspects of plant biomass while it had no effect on defoliation damage, growth rate, or root to shoot ratio. The random effect of plant subject explained <20% of the variation in these latter response variables. On the other hand, plant subject explained 40-60% of the variation in biomass attributes.

The latitude*treatment and introgression*treatment interactions were not significant for defoliation damage (Appendix 1), indicating no difference in damage either across latitude or species introgression. However, there was a significant effect of treatment type on plant damage ($F = 120$, $df = 2, 77.6$, $P < 0.0001$). Damage from the chemical treatment (LS mean ± SE: 63.9 ± 2.6%, range 20-93%) was greater than damage from the herbivore treatment (LS mean ± SE:

35.1 ± 2.6%, range 3-94%). Both chemical and herbivore treatments resulted in significantly greater plant damage than the control treatment (LS mean ± SE: 7.5 ± 2.6%, range 1-32%).

My proxy for plant fitness, annual canopy growth rate, also was not affected by either latitude or species introgression (Appendix 1). Defoliation treatment had a significant effect on this response variable ($F = 120$, $df = 2, 77.6$, $P < 0.0001$). Average fitness scores among the treatments were 37.7 ± 5.0%, 33.3 ± 5.0%, and 11.63± 6.1%, for control, herbivore, and chemical defoliation, respectively. Fitness scores ranged between -100.0 and 93.6% annual canopy growth. Six of the 43 clones assigned to the chemical treatment died during the winter following the treatment application (fitness = -100% canopy growth). These plants were retained in the analysis of tolerance to defoliation (see below).

Plant biomass had a negative trend with species introgression ($F = 4.7$, $df = 1, 39.7$, $P = 0.04$). Control plants with high levels of *T. ramosissima* introgression had lower biomass than plants with low levels of *T. ramosissima* introgression (Fig. 3.2). In addition, defoliation treatment had a significant effect on plant biomass ($F = 16.6$, $df = 2, 74.5$, $P <0.0001$) but the interaction between treatment and introgression was not significant. Defoliation by either chemical or herbivore treatments led to significantly reduced coarse root and woody stem biomass (Fig. 3.3). On the other hand, only plants that were chemically defoliated had significantly lower green foliage and fine root biomass compared to control plants.

Evidence ratios of AIC weights indicated that the introgression model was more likely to be the best model versus the latitude model at predicting root to shoot ratios (Table 3.3). Results of ANCOVA with introgression as a dependent variable expose both a significant treatment effect ($F = 8.5$, $df = 2,78.6$, $P < 0.001$) and a significant effect of species introgression ($F = 4.5$, $df = 1,40.5$, $P = 0.04$) but not a significant interaction between these two factors. Plants in the

herbivore (LS mean: 0.29 ± 0.02) and control (LS mean: 0.27 ± 0.02) treatment groups both had significantly greater root:shoot ratios than plants in the chemical treatment (LS mean: 0.19 ± 0.02) but were not significantly different than each other. Introgression showed a positive relationship with root to shoot ratios (ratio = 0.00116*introgression + 0.20), implying that plants with high levels of *T. ramosissima* introgression invest more in belowground rather than aboveground growth.

Tolerance

I defined tolerance as the slope of the linear relationship between damage and fitness for each of the 43 genotypes (Appendix 2). These genotypes, which were haphazardly chosen from the larger pool of 342 plants, had a mean species introgression level of 58.0 ± 3.4% *T. ramosissima* (range 20.5-96.2%). Tolerance demonstrated a positive relationship with introgression using weighted regression analysis (tolerance = 0.004*introgression – 0.40; $F = 6.27$, $df = 1,41$, $P = 0.02$). Plants with high levels of *T. ramosissima* introgression had high tolerance to defoliation (Fig. 3.4). Since models investigating introgression and latitude could not be distinguished (see above), I also analyzed tolerance versus latitude. There was a significant positive relationship of latitude with tolerance (tolerance = 0.09*latitude – 3.7; $F = 25.1$, $df = 1,41$, $P < 0.0001$; $R^2 = 0.38$).

Resistance

Resistance to *D. carinulata* was determined with the plant pellet bioassay. High resistance to herbivory was reflected by a small proportion, p, of pellet consumed. There was a negative relationship between species introgression and resistance (Fig. 3.5A). Pellets made from plants with high levels of *T. ramosissima* were consumed more in these no-choice trials. Additionally, larvae used in this experiment gained more mass when fed pellets with high *T.*

ramosissima introgression (Fig. 3.5B), confirming the low resistance of these plant genotypes. Latitude also showed a negative relationship with resistance (resistance = 1.2 – 0.01*latitude; F = 11.2, df = 1, 28, P = 0.002; R^2 = 0.30) and positive relationship with larval performance (larval performance = 0.65*latitude – 32.3; F = 5.3, df = 1, 26, P = 0.03; R^2 = 0.30).

DISCUSSION

I examined the relationship among species introgression, latitude of population origin, and various plant performance traits of the invasive shrub tamarisk (*Tamarix* sp.). Two previous studies showed that *T. ramosissima*-backcrossed hybrids are common in northern U.S. latitudes and *T. chinensis*-backcrossed hybrids are common in southern U.S. latitudes (Friedman et al. 2008, Gaskin and Kazmer 2009), but no quantitative description between latitude and hybridization has been reported. Here I report a positive linear relationship between latitude and *T. ramosissima* species introgression. For tamarisk sites in the western U.S. between latitudes 32.0 and 46.7 ^{o}N, every one degree increase in latitude corresponds to a 4.6% positive change in *T. ramosissima* introgression among tamarisk populations (Fig. 3.1). Additionally, *T. ramosissima* parental genotypes only occurred in northern populations (41.3-47.6 ^{o}N) while *T. chinensis* parental genotypes were recorded from southern latitudes (32.0-37.1 ^{o}N). The exact cause of the latitudinal cline in tamarisk hybridization is unknown. One possible mechanism is that hybridization occurred in nurseries in the early 1800s before tamarisk was introduced at multiple locations in the western U.S. (Gaskin et al. 2012) while others have suggested that several independent hybridization events could have occurred in areas where both parent species were co-introduced (Friedman et al. 2008, Gaskin and Kazmer 2009, Friedman et al. 2011). Under either scenario, subsequent sorting out of adapted hybrid genotypes then occurred post-introduction. Similar patterns of latitude and genetic variation are correlated with at least two

tree species in their native range (Ledig 2000, Marchelli and Gallo 2001) and the perennial shrub, *Eupatorium adenophorum*, in its invasive range (Huang et al. 2009).

Adaptation appears to be an important evolutionary mechanism for hybrid tamarisk populations in terms of particular life history traits (e.g. cold hardiness and leaf phenology; Friedman et al. 2008, 2011), but whether variation in these traits can be explained by levels of species introgression versus latitude of population origin remain unknown. In the current study, I employed Akaike's Information criterion (AIC) to determine which factor, latitude or species introgression, better explains variation in plant performance traits (Burnham and Anderson 2002). While biomass and herbivore defense traits were equally explained by either predictor variable, growth rate was slightly more influenced by latitude than introgression although this factor was not significant when tested using ANCOVA. Root-to-shoot ratios were more affected by introgression versus latitude (Table 3.3), and this relationship was statistically significant. However, given the high correlation between latitude and introgression for the populations in this study, future experiments would be required to tease these two factors apart.

In order to determine the relative importance of latitudinal clines and hybrid status on tamarisk life history traits, quantitative trait loci mapping and breeding experiments involving parent species to produce F1, F2, and backcrossed offspring are necessary (Cheng et al. 2011). Additionally, comparing parent species from the native range versus parental and hybrid genotypes from the introduced range would reveal how important hybridization is in this system (Keller and Taylor 2008, Ridley and Ellstrand 2010). In terms of assessing the strength of latitudinal clines, caution should be taken when drawing inferences from a single common garden experiment (Williams et al. 2008, Colautti et al. 2009). Multiple common gardens with plants collected from a wide range of latitudes in both ranges are ideal for determining whether

evolution of latitudinal clines has occurred (Maron et al. 2004, Colautti et al. 2009). Given that a study which combines both latitude and hybridization may be difficult, perhaps focusing attention on U.S. populations that naturally possess a wide range of genetic variability could be fruitful. For instance, variation in species introgression was highest among plants from Cimarron River (4.0-75.5% *T. ramosissima*; Table 3.1). Common garden studies and feeding trials involving *D. carinulata* on plants from this population may shed more light on the applied implications of hybridization.

My next goal was to describe the relationships between hybridization and/or latitude and various plant performance traits. In the outdoor common garden experiment, latitude and introgression both significantly affected biomass, while root-to-shoot ratios were affected by introgression but not latitude. Canopy growth rate was not affected by either factor. Latitudinal clines in biomass, plant height, shoot number, flowering time and fecundity have been recorded in a number of invasive plant species in their introduced ranges (Weber and Schmid 1998, Kollman and Bañuelos 2004, Maron et al. 2004, Leger and Rice 2007, Montague et al. 2008). Here, plants from southern populations and plants with less *T. ramosissima* introgression grew larger, especially in terms of woody stems. Additionally, plants with higher *T. ramosissima* introgression allocated more resources to belowground tissues versus aboveground tissues. Sexton et al. (2002) observed variation in root-to-shoot ratios of tamarisk populations growing in a common environment and attributed these observations to climatic differences at latitudes of origin. Friedman et al. (2008) reasoned that plants from northern populations suffered more dieback as the result of extreme low temperatures, thus these hybrids evolved increased cold hardiness and belowground tissue allocation. The fact that introgression explained a significant amount of variation in root-to-shoot ratios could be an example of evolutionary novelty where

hybrid success is explained by recombination of traits from both parents (Ellstrand and Schierenbeck 2000).

Belowground tissues store carbohydrates, which provide necessary energy required for tamarisk leaf flush following defoliation (Hudgeons et al 2007). In my study, plants with high levels of *T. ramosissima* introgression were more tolerant of defoliation than plants with low levels of *T. ramosissima* introgression (Fig. 3.4). This result is congruent with the finding that northern plants (i.e., plants with high *T. ramosissima* introgression) invest more in belowground growth than aboveground growth. *T. ramosissima* genotypes may have adapted strategies to cope with *D. carinulata* outbreaks in the native range (Lewis et al. 2003). In the introduced range, *T. chinensis*-dominated hybrids appear susceptible to control strategies involving defoliation of photosynthetic tissue. For instance, *D. carinulata* has caused the most impact on tamarisk populations whose introgression values are <60% *T. ramosissima* (DeLoach et al. 2009, Gaskin and Kazmer 2009). However, *D. carinulata* has failed to establish at northern U.S. sites where *T. ramosissima* introgression is high, making comparisons of biological control efficacy problematic. In these northern locations, establishment of the biological control agents may be attributed to abiotic factors acting on beetle populations (Lewis et al. 2003, Herrera et al. 2005, Milbrath et al. 2007). Nonetheless, tolerance scores differed across introgression in the common garden. Given that tolerance is an herbivore defense strategy that has a strong degree of inheritance (Strauss and Agrawal 1999, Agrawal et al. 2004), hybridization between *T. ramosissima* and *T. chinensis* could possibly affect biological control in the field.

My study also revealed an interesting ecological relationship between defoliation and plant performance, which may have implications in an applied sense. Only a modest amount of damage by beetles (mean = 35.1%) significantly reduced annual growth of woody stems and

coarse roots compared to control plants (mean damage = 7.5%). Moreover, this moderate amount of defoliation had the same effect on woody stems and coarse roots as did a larger amount of damage (mean = 63.9%) by chemical defoliation (Fig. 3.3). Therefore, *D. carinulata* appears to reduce allocation to these structural and storage tissues as much as chemical defoliation. Indeed, herbivore defoliation reduces carbohydrate stores in roots, eventually contributing to mortality of tamarisk shrubs (Hudgeons et al. 2007, Pattison et al. 2011). On the other hand, chemical defoliation by carfentrazone-ethyl significantly reduced growth of photosynthetic tissue and fine roots the following year while defoliation by herbivores did not. Chemical control appears to have a more immediate effect in terms of overall plant size compared to defoliation by the biological control agent. Of course, this logic is based on the amount of defoliation attributed to either chemical or herbivore treatment in my experiment. Actual defoliation by either carfentrazone-ethyl or *D. carinulata* may be lower or higher in field locations, especially given the fact that *D. carinulata* completes 2-4 generations per year in the U.S. (Lewis et al. 2003).

In terms of resistance to herbivory, there were no significant differences among populations or hybrid genotypes for amount of canopy damaged in the outdoor garden experiment (Appendix 1). However, in the bioassay experiment there were significant effects of tamarisk hybridization on interactions with the biological control agent. Resistance to herbivory decreased with increasing amounts of *T. ramosissima* introgression (Fig. 3.5A) and larvae that were fed pellets made from plants with high *T. ramosissima* introgression gained more mass (Fig. 3.5B). The fact that there were genetic differences in the bioassay experiment versus the outdoor experiment could be due to the response of tamarisk plants to *D. carinulata* feeding. Both larvae and adults are adapted to chew on photosynthetic stems, oftentimes girdling them. The response of the plants is to abscise the entire stem and leaf even if the majority of the tissue

is undamaged (Pattison et al. 2011, pers obs,). Thus, beetles in the outdoor experiment could have preferentially fed upon various tamarisk hybrids, but the amount of herbivory was masked by this abscission. Several accessions and species of tamarisk have been tested in host selection trials, oftentimes with significant differences in attack or insect development (DeLoach et al. 2003, Lewis et al. 2003, Milbrath and DeLoach 2006, Dalin et al. 2009, Moran et al. 2009). The current study quantifies the relationship between tamarisk hybridization and resistance to herbivores.

Previous studies have reported differences in resistance among hybrid genotypes (Fritz et al. 1998, Craig et al. 2000, McGuire and Johnson 2006, Krebs et al. 2011). A possible mechanism for these differences is novel chemistry of hybrid host plants (Orians 2000). Herbivorous insects use plant chemistry as a means of host recognition while plants employ a variety of compounds for defense against herbivores (Schoonhoven et al. 2005). Biochemical novelty due to plant hybridization can arise in at least three different ways: obstruction of biosynthetic pathway leading to the production of different secondary compounds; enzymes inherited from one parent modifying compounds whose pathways were inherited from the second parent; and changes in regulatory genes leading to zero production of certain compounds or producing the chemical cues in different tissues (Cheng et al. 2011). Indeed, genotypic differences in secondary compounds can be significant within a hybrid class (Cheng et al. 2011). Three reviews of more than 30 studies involving over 80 taxa show that 5.5% of secondary compounds of hybrid genotypes are novel compared to either parent species while nearly 20% of hybrid plants either over- or under-express secondary compounds compared to their parents. Moreover, 5.9% of hybrids have higher resistance to herbivory compared to parent species (Rieseberg and Ellstrand 1993, Orians 2000, Cheng et al. 2011). While plant-based chemical

attractants are known for *D. carinulata* (Cosse et al. 2006), there is no information whether these chemicals (green leaf volatiles) differ among hybrid genotypes. Furthermore, there are no other published records of tamarisk chemistry in the introduced range. It would be interesting to see if differences in tamarisk resistance to herbivory can be explained by differences in production of secondary metabolites.

The high percentage of hybrid tamarisk genotypes relative to parental genotypes in the western U.S. underscores the importance of hybridization to plant invasion (Campbell et al. 2006, Ridley and Ellstrand 2010, Whitney et al. 2006). Using a similar Bayesian cluster analysis of AFLP data on 110 plants collected from a large geographical area in the western U.S., Gaskin and Kazmer (2009) found that hybrids make up 87% of genotypes in the invasion. They were only able to detect four parental *T. ramosissima* genotypes and ten parental *T. chinensis* genotypes. In the current study, hybrids made up 88% of the 342 plants sampled with 37 parental *T. ramosissima* and four parental *T. chinensis* documented. Another study documenting the success of hybrid tamarisk in the western U.S. comes from Gaskin et al. (2012). In their study, 180 tamarisk plants ranging from 1-70+ years of age were sampled along a 70-km stretch of the Green River in southern Utah. Using clustering analysis of AFLP data, they found no difference in species introgression across tamarisk age class. Moreover, they failed to detect any parental genotypes in the study area with species introgression ranging from 28.3-73.3% *T. ramosissima*. They conclude that hybridization either occurred pre-invasion or parental genotypes have been replaced altogether on the landscape (Gaskin et al. 2012). Although there are no tests of selective advantages of tamarisk hybrids over parents in the new range, the high ratio of hybrids to parental genotypes (>5:1) suggests that such an advantage exists.

Rapid evolution of life history traits is common in introduced plants and often is invoked as a mechanism of invasiveness (Ellstrand and Schierenbeck 2000). Hybridization and latitudinal clines have recently been deemed commonplace in invasive plant populations (Schierenbeck and Ellstrand 2009, Hodgins and Rieseberg 2011). While recording evolved differences in size and fecundity, researchers should also investigate whether these factors affect biological control. Some plant hybrids may be more susceptible to herbivory while others more resistant. If hybrids are spread across the landscape in a predictable manner, as is the case with *Tamarix* invasion in the western U.S., this knowledge regarding the response of particular hybrids to herbivory can be exploited to maximize biological control efforts. Hybridization and adaptation of invasive plants provide valuable opportunities to study evolution for both basic and applied significance.

Table 3.1. Latitude, longitude, and elevation of sites where Friedman et al. (2008) made original collections. Minimum, maximum, and mean species introgression with standard error calculated as percentage *T. ramosissima* based on AFLP genetic fingerprinting and subsequent assignment analysis of 342 total plants. *, Populations used in the outdoor garden experiment; †, Populations used in the bioassay experiment; ‡, Location of the outdoor common garden.

Population	Lat(°N)	Lng(°W)	El(m)	Min	Max	Avg	SE	n
Colorado River, TX†	32.020	-100.737	579	6.5	35.6	22.7	1.7	23
Lake Alan Henry, TX*	33.063	-101.042	648	11.1	43.1	24.7	1.7	22
Buffalo Lake, TX*	34.904	-102.118	1106	20.5	66.7	42.3	2.7	22
Lake Meredith, TX*	35.529	-101.767	897	9.7	54.7	30.6	2.2	24
Cimarron River, OK†	37.122	-101.892	1031	4.0	76.6	46.2	3.5	25
Arkansas River, CO*	38.087	-102.288	1056	36.2	72.4	54.2	1.8	25
Bonny Reservoir, CO*	39.623	-102.194	1121	20.3	70.1	44.1	2.8	22
Poudre River, CO*	40.559	-105.015	1489	45.1	91.0	71.9	2.0	32
Lake McConaughy, NE*	41.291	-101.933	999	26.1	85.8	60.9	2.7	25
Boysen Reservoir, WY*	43.222	-108.180	1443	65.7	97.5	82.1	2.5	26
Keyhole Reservoir, WY†	44.368	-104.792	1251	62.7	96.2	82.4	1.7	25
Powder River, MT*	45.427	-105.405	923	67.1	93.3	78.9	1.5	24
Musselshell River, MT†	46.445	-108.525	967	76.8	97.9	92.9	1.1	25
Fort Peck Reservoir, MT*	47.604	-106.902	686	68.9	97.1	83.8	1.8	22
Fort Collins, CO‡	40.573	-105.082	1529			-	-	-

Table 3.2. Competing models used in AIC model selection. Model 1 investigates the influence of latitude while Model 2 focuses on species introgression. Plant subject was modeled as a random effect while the other factors were considered fixed effects. *Only these factors were included in models investigating resistance in the bioassay experiment.

Factors in model	Model 1	Model 2
Defoliation treatment	X	X
Latitude*	X	
Treatment x Latitude	X	
Introgression*		X
Treatment x Introgression		X
Block	X	X
Plant subject*	X	X
Initial plant size	X	X

Table 3.3. AICc results of competing models for six response variables. A lower AICc score for a given response variable indicates that more variation is explained using that particular model than its competitor. Differences in AICc scores (Δ_i) between a competing model and the best fit model is an indication of model selection. A Δ_i, < 2 indicates no clear best model, $4<\Delta_i$, <7 indicates less evidence for a competing model, while Δ_i, >10 means there is essentially no support for the competing model. Akaike weights (w_i) are used to calculate evidence ratios. An evidence ratio of three means that there is relatively little evidence for that model.

Dependent variable	Model	AICc	Δ_i	w_i	Evid.ratio
Defoliation damage	1	1115.3	0.7	0.41	1.4
	2	1114.6	0	0.59	1.0
Canopy growth	1	1279.4	0	0.88	1.0
	2	1283.4	4.0	0.12	7.4
Biomass	1	1019.4	1.1	0.37	1.7
	2	1018.3	0	0.63	1.0
Root:shoot	1	-149.4	4.6	0.09	10.0
	2	-154.0	0	0.91	1.0
Pellet resistance	1	827.7	3.1	0.18	4.7
	2	824.6	0	0.82	1.0
Larval mass	1	804.0	2.4	0.23	3.3
	2	801.6	0	0.77	1.0

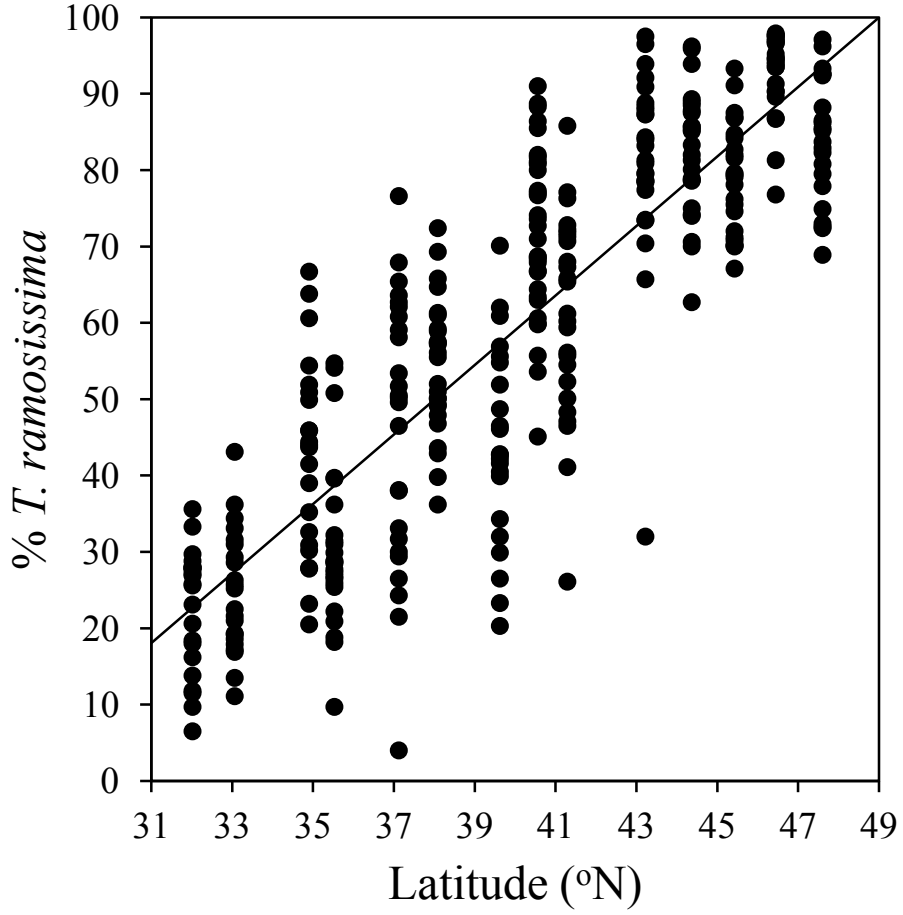


Figure 3.1. The relationship between tamarisk species introgression, expressed as percentage *T. ramosissima*, versus latitude of population origin (%*T. ramosissima* introgression = 4.6*latitude - 123; $R^2 = 0.74$; n=342). See Table 3.1 for detailed site descriptions.

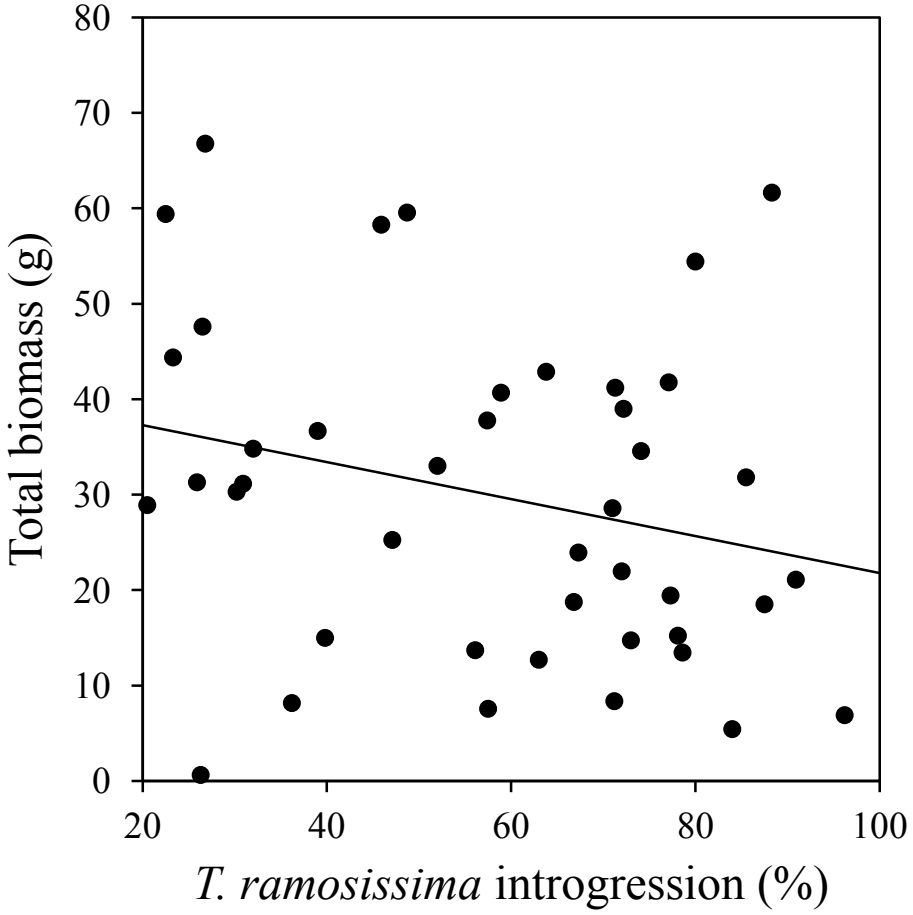


Figure 3.2. The effect of species introgression on total biomass at the end of the experiment. This figure depicts the univariate relationship between introgression and biomass for only the plants in the control treatment (n=43). In the full model, biomass = 21.0 -0.17*introgression ($F = 4.7$, $df = 1,39.7$, $P = 0.04$; $R^2 = 0.78$).

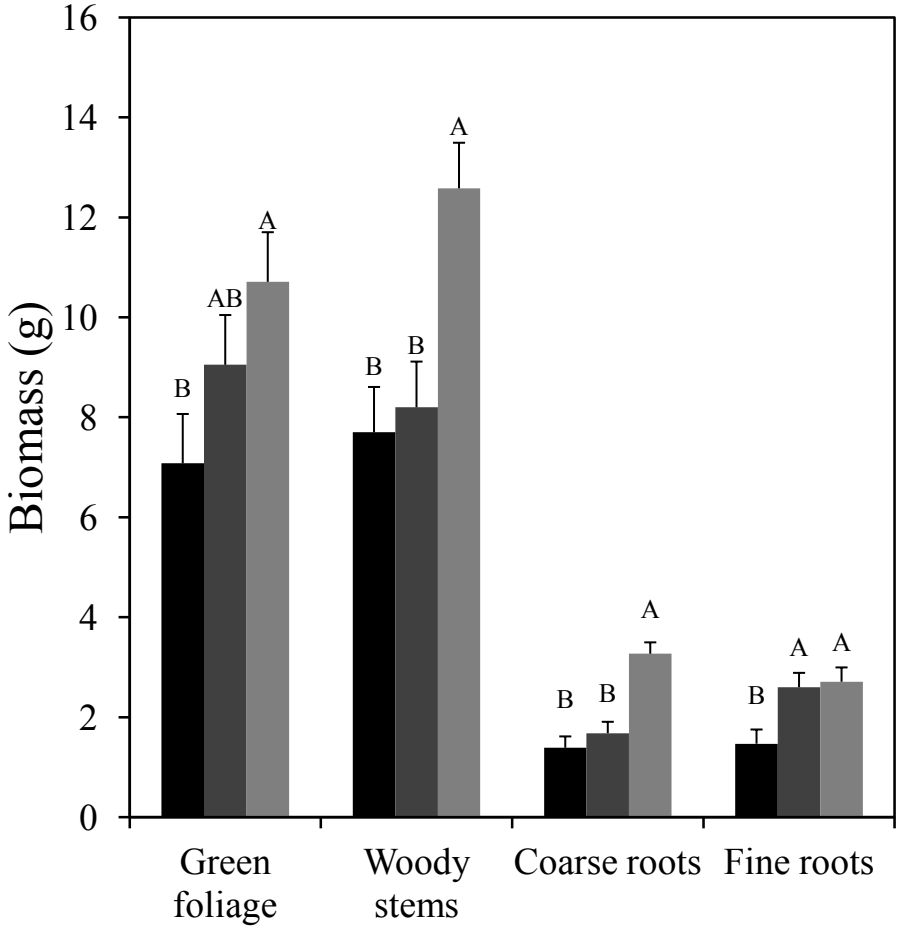


Figure 3.3. LS mean biomass ± SE of four plant tissue groups for three defoliation treatments. Chemical: black bars; herbivore: dark grey bars; control: light grey bars. Different letters correspond to differences among means within a plant tissue category (Student's t-test, $\alpha = 0.05$). Defoliation treatments were applied in the summer of 2010 and plant parts were harvested, dried and weighed the following spring. Mean percent canopy defoliation was 64%, 35%, and 7.5% for chemical, herbivore, and control treatments, respectively.

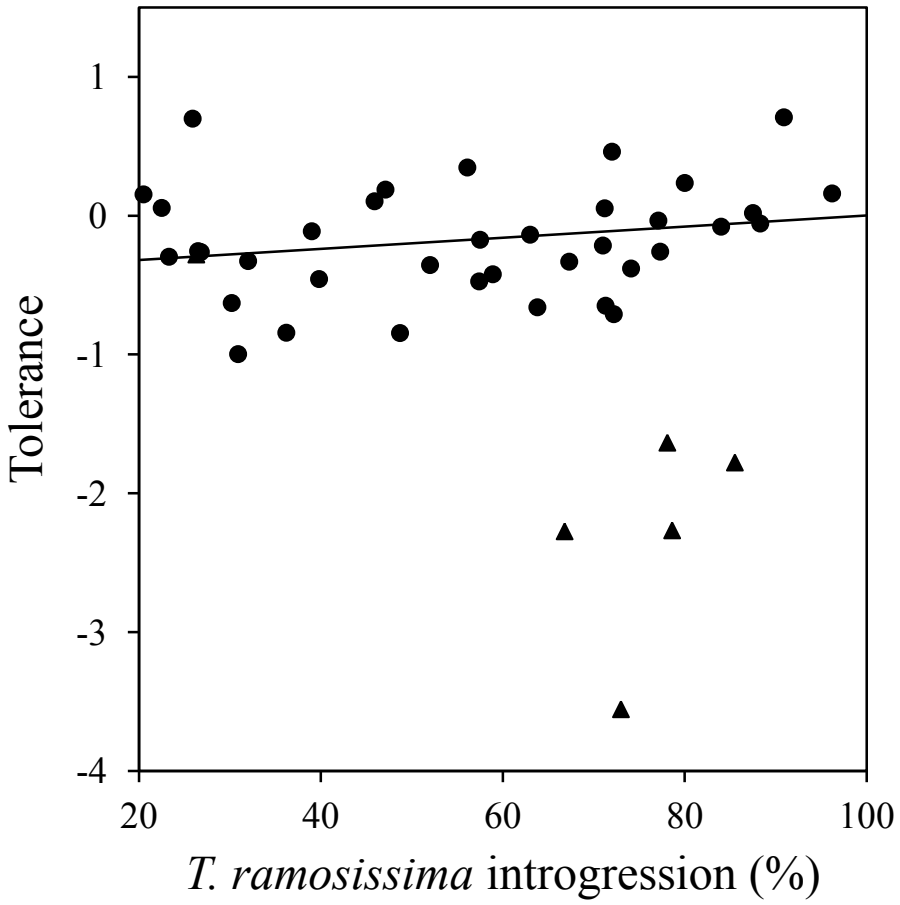
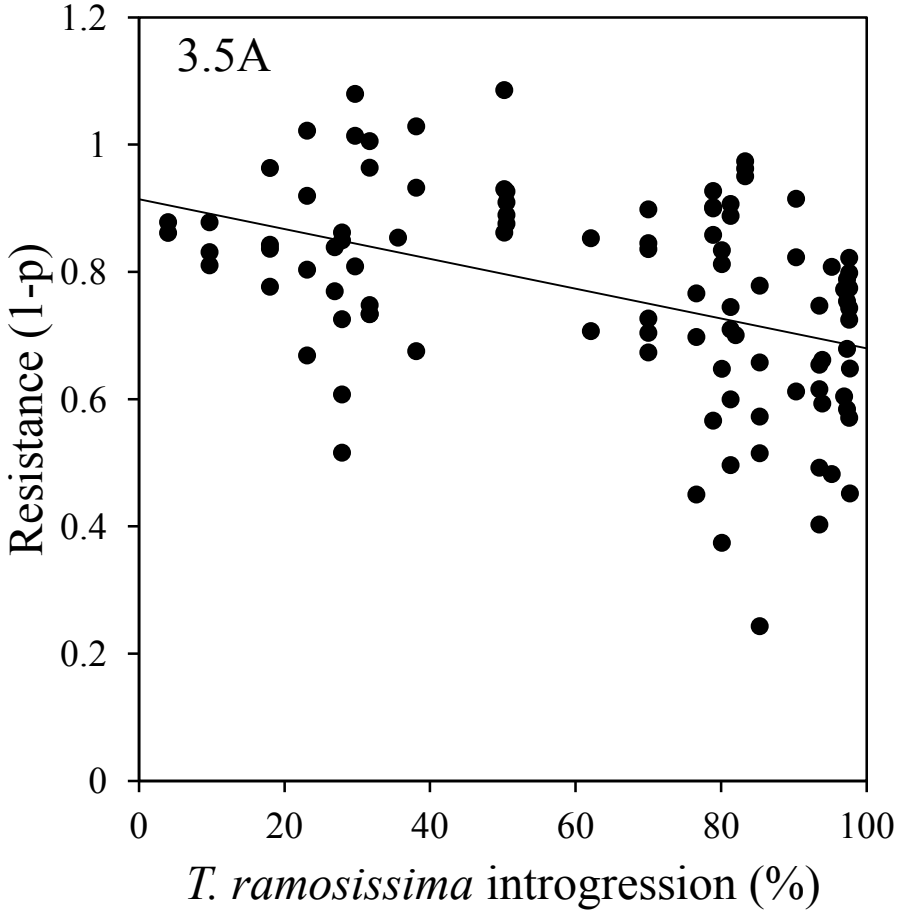


Figure 3.4. Tolerance to defoliation across species introgression for plant genotypes in the experiment. Positive tolerance values correspond to plant over-compensation while negative values correspond to plant under-compensation to defoliation. Tolerance demonstrated a positive relationship with introgression when the SE's of tolerance scores were included in a weighted regression (tolerance = 0.004*introgression – 0.40; $R^2 = 0.13$). Weighted regression was used because some clones in the chemical treatment died, thus leading to more error for those particular genotypes (triangles). See Appendix 2 for detailed tolerance scores of each genotype.


3.5A
Resistance (1-p)
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
T. ramosissima introgression (%)

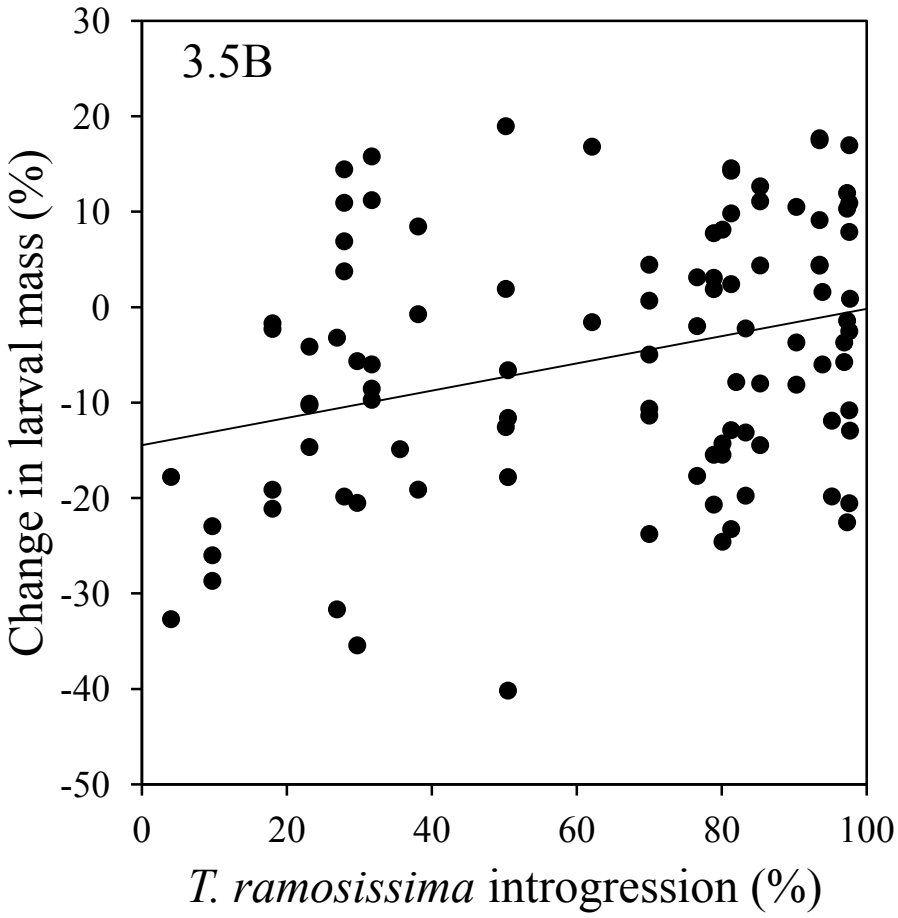


Figure 3.5. Plant genotype resistance to herbivory by *D. carinulata* in the pellet bioassay (3.5A). Resistance is defined as 1-proportion of pellet damage. Pellets made from plants with high levels of *T. ramosissima* introgression had greater resistance (resistance = 0.919 – 0.002*introgression; F = 15.2, df = 1,28.9, P < 0.001; $R^2 = 0.42$). Percent change in larvae mass in the pellet bioassay (3.5B). Plant species introgression had a negative effect on larval performance (%Change in larval mass = 0.15*introgression – 15.2; F = 8.0, df = 1,27.8, P < 0.01; $R^2 = 0.29$).

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Appendix 1. ANCOVA results for latitude (1A) and introgression (1B). Response variables included plant damage from the defoliation treatments, canopy growth rate (estimate of plant fitness), biomass at the end of the experiment, and the ratio of belowground to aboveground growth for plants in the outdoor garden experiment. Pellet damage and larval mass refer to the plant genotype resistance and larval performance, respectively, in the pellet bioassay experiment. Plant variation refers to the amount of variation explained by the random variable in the model, plant subject.

1A:

Response variable	R^2	Treatment *F*(df)	Latitude *F*(df)	T x L *F*(df)	Block *F*(df)	Initial size *F*(df)	Plant (%)
Defoliation damage	0.70	120(2,77.6)***	0.14(1,41.0)	0.09(2,79.4)	2.8(6,115)*	1.5(1,84.3)	2.5
Canopy growth rate	0.46	9.9(2,74.6)***	0.04(1,39.1)	2.3(2,75.8)	1.6(6,109)	0.87(1,101)	18.8
Total biomass	0.77	16.6(2,74.5)***	5.5(1,40.8)*	1.4(2,74.4)	1.4(6,89.9)	19.3(1,113)***	53.5
Aboveground	0.77	13.5(2,74.7)***	6.0(1,40.9)*	1.5(2,74.6)	1.7(6,90.3)	16.8(1,113.1)***	53.0
Green foliage	0.68	6.5(2,75.9)**	2.3(1,41.9)	0.81(2,75.9)	1.6(6,93.8)	6.4(1,115)*	47.1
Woody stems	0.82	20.9(2,73.6)***	8.8(1,40.1)**	4.1(2,73.5)*	1.4(6,86.9)	27.6(1,110)***	58.9
Belowground	0.72	22.2(2,74.3)***	3.1(1,40.2)†	0.82(2,74.3)	0.81(6,94.4)	20.3(1,115)***	43.7
Fine roots	0.67	11.6(2,76.4)***	1.8(1,42.2)	0.94(2,76.5)	1.6(6,97.6)	18.2(1,115)***	40.0
Coarse roots	0.73	32.9(2,73.6)***	3.4(1,38.6)†	0.71(2,72.6)	0.57(6,93.1)	13.1(1,115)***	44.2
Roots:Shoots	0.44	8.1(2,78.9)***	2.5(1,43.1)	0.05(1,79.8)	5.0(6,112)***	0.13(1,97.7)	11.3
Pellet resistance	0.42	NA	11.2(1,28.0)***	NA	NA	NA	22.9
Larval mass	0.30	NA	5.3(1,26.0)*	NA	NA	NA	16.2

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$

1B:

Response variable	R^2	Treatment *F*(df)	Introgr. *F*(df)	T x I *F*(df)	Block *F*(df)	Initial size *F*(df)	Plant (%)
Defoliation damage	0.71	121.3(2,77.8)***	0.23(1,39.2)	0.39(2,78.3)	3.1(1,115)**	1.9(1,83.5)	2.5
Canopy growth rate	0.43	9.4(2,74.5)***	0.06(1,36.7)	0.40(2,74.6)	2.0(6,109)	0.77(1,97.5)	17.3
Total biomass	0.78	17.0(2,75.2)***	4.7(1,39.7)*	2.2(2,74.7)	1.3(6,89.7)	22.2(1,113)***	55.3
Aboveground	0.77	13.9(2,75.3)***	4.9(1,39.8)*	2.5(2,74.8)†	1.6(6,89.9)	19.7(1,113.1)***	55.2
Green foliage	0.70	6.7(2,76.0)**	1.3(1,40.2)	2.2(2,75.5)	1.7(6,92.9)	7.3(1,115)**	49.4
Woody stems	0.82	21.2(2,74.7)***	8.8(1,39.4)**	4.3(2,74.2)*	1.1(6,87.5)	32.2(1,111)***	59.7
Belowground	0.72	22.4(2,75.0)***	3.3(1,40.0)†	1.0(2,74.5)	0.67(6,94.5)	22.7(1,115)***	44.6
Fine roots	0.68	11.8(2,76.7)***	1.7(1,40.4)	1.4(2,76.2)	1.4(6,97.3)	19.9(1,115)***	41.0
Coarse roots	0.73	32.9(2,73.7)***	4.0(1,37.6)†	0.52(2,73.2)	0.52(6,93.7)	15.2(1,115)***	44.4
Roots:Shoots	0.45	8.5(2,78.6)***	4.5(1,40.5)*	1.2(1,78.4)	5.3(6,112)***	0.40(1,95.5)	10.5
Pellet resistance	0.42	NA	15.2(1,28.9)***	NA	NA	NA	19.4
Larval mass	0.29	NA	8.0(1,27.8)**	NA	NA	NA	14.3

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$

Appendix 2. Tolerance scores for each of the 43 genotypes in the outdoor garden study. Tolerance refers to the slope and SE of the linear relationship between percent canopy defoliation and fitness. A negative slope shows under-compensation for defoliation while a positive slope indicates over-compensation. A significant P value for a t ratio indicates that the slope is significantly different from zero. All P values <0.1 are shown in bold. The latitude of origin and species introgression for each plant subject is provided. *, indicates that the clones for these particular genotypes assigned to the chemical treatment died and thus their tolerance scores were influenced. This influence was reflected in the SE weights, where a low weight corresponds to less influence in the regression (Fig. 3.4).

Genotype	Latitude	Introgression	Tolerance	SE	SE weights	t ratio	P value
421	33.063	36.2	-0.843	0.103	93.7	-8.17	**0.08**
556	33.063	22.5	0.056	0.307	10.6	0.18	0.89
602*	33.063	26.3	-0.281	1.917	0.3	-0.15	0.91
122	34.904	39.0	-0.113	0.241	17.2	-0.47	0.72
187	34.904	30.9	-0.997	0.417	5.7	-2.39	0.25
349	34.904	45.9	0.103	2.450	0.2	0.04	0.97
463	34.904	20.5	0.153	0.126	63.4	1.22	0.44
471	34.904	30.2	-0.630	0.100	99.4	-6.27	0.10
591	34.904	63.8	-0.660	0.499	4.0	-1.32	0.41
481	35.529	25.9	0.699	0.992	1.0	0.70	0.61
514	35.529	26.5	-0.256	0.214	21.9	-1.20	0.44
561	35.529	26.8	-0.262	0.290	11.9	-0.90	0.53
86	38.087	52.0	-0.356	0.046	475.6	-7.76	**0.08**
302	38.087	57.5	-0.174	0.136	54.2	-1.28	0.42
411	38.087	57.4	-0.474	0.198	25.4	-2.39	0.25
488	38.087	56.1	0.347	0.443	5.1	0.78	0.58
502	38.087	58.9	-0.422	0.229	19.0	-1.84	0.32
644	38.087	39.8	-0.457	0.225	19.7	-2.03	0.29
41	39.623	23.3	-0.296	0.007	22306.4	-44.23	**0.01**
636	39.623	32.0	-0.327	0.039	663.0	-8.42	**0.08**
671	39.623	48.7	-0.846	0.100	99.2	-8.42	**0.08**
107	40.559	77.3	-0.260	0.303	10.9	-0.86	0.55
110*	40.559	66.8	-2.274	0.102	96.9	-22.38	**0.03**
166	40.559	63.0	-0.137	0.173	33.2	-0.79	0.57
243	40.559	88.3	-0.057	0.055	324.8	-1.03	0.49
261	40.559	71.0	-0.215	0.260	14.8	-0.83	0.56
352	40.559	74.1	-0.381	0.046	472.9	-8.28	**0.08**
649	40.559	80.0	0.235	0.290	11.9	0.81	0.57
46	41.291	72.2	-0.710	0.283	12.5	-2.51	0.24
139	41.291	71.3	-0.649	0.047	443.8	-13.67	**0.05**
149	41.291	77.1	-0.036	0.217	21.2	-0.16	0.90
645	41.291	47.1	0.187	0.107	87.9	1.76	0.33
672	41.291	67.3	-0.332	1.208	0.7	-0.27	0.83
548	43.222	90.9	0.708	0.096	108.3	7.37	**0.09**

583	43.222	84.0	-0.080	0.026	1517.8	-3.10	0.20
643*	43.222	78.6	-2.267	0.530	3.6	-4.28	0.15
30	45.427	87.5	0.019	0.285	12.3	0.07	0.96
231	45.427	72.0	0.460	0.030	1086.8	15.18	**0.04**
270*	45.427	78.1	-1.636	1.764	0.3	-0.93	0.52
449	45.427	71.2	0.053	0.123	65.9	0.43	0.74
239	47.604	96.2	0.160	0.148	45.4	1.08	0.48
240*	47.604	85.5	-1.778	0.423	5.6	-4.21	0.15
244*	47.604	73.0	-3.555	0.773	1.7	-4.60	0.14