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PHYSIOLOGICAL RESPONSE OF *TAMARIX RAMOSISSIMA* (TAMARICACEAE) TO A BIOLOGICAL CONTROL AGENT

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ABSTRACT.—Within the last century, the floristic composition of riparian communities in the Southwest has drastically changed following introduction of the exotic tree *Tamarix ramosissima.* In an attempt to control *Tamarix* populations, the tamarisk leaf beetle (*Diorhabda carinulata*) has been utilized as a biological control agent. Three years of data collection at our study sites along Fountain Creek (Fountain, CO) allowed us to characterize the response of *Tamarix* to invasion by the biological control agent. In analyzing data collected before, during, and after the beetle invasion, we observed a significant effect of foliar herbivory on *Tamarix* physiology and life history strategy. Associations between flower number and functional traits changed before, during, and after the beetle invasion. Before the invasion, reproductively fit individuals exhibited high stomatal conductance and used relatively more water. During and after the invasion, fit plants had higher foliar chlorophyll content, but conductance was not significantly correlated with fecundity. *Tamarix* responded to defoliation by increasing water use, which may have been an attempt to sustain photosynthate allocation to reproductive structures. Therefore, the leaf beetle may increase the water use of *Tamarix* during the growing season.

RESUMEN.—Durante el último siglo, la composición floral de las comunidades ribereñas en el suroeste ha cambiado drásticamente tras la introducción del árbol exótico *Tamarix ramosissima.* En un intento por controlar las poblaciones de *Tamarix,* se ha utilizado al escarabajo de la hoja de tamariscos (*Diorhabda carinulata*) como control biológico. La colecta de datos durante tres años en nuestras áreas de estudio a lo largo de Fountain Creek (Fountain, Colorado) nos permitió caracterizar la respuesta de *Tamarix* a la invasión del agente de control biológico. Al analizar los datos obtenidos antes, durante y después de la invasión del escarabajo, observamos un efecto significativo de la herbivoría foliar en la fisiología y la estrategia de historia de vida de *Tamarix.* Las asociaciones entre el número de flores y los rasgos funcionales se modificaron antes, durante y después de la invasión de escarabajos. Antes de la invasión, los individuos reproductivamente aptos mostraban en los estomas una conductancia elevada y utilizaban relativamente más agua. Durante y después de la invasión, las plantas sanas mostraron un mayor contenido de clorofila foliar, pero la conductancia no se correlacionó significativamente con la fecundidad. El *Tamarix* respondió a la defoliación mediante el aumento en el uso de agua, lo que pudo ser un intento para mantener la asignación de fotosintatos a las estructuras reproductivas. Por lo tanto, el escarabajo puede aumentar el uso del agua de *Tamarix* durante su estación de crecimiento.

Species of the nonnative genus *Tamarix* (Tamaricaceae) are invasive trees or shrubs within riparian ecosystems of the southwestern United States (Thomaso 1998, DeLoach et al. 2003). *Tamarix* species aggressively invade riparian ecosystems and can completely exclude native species by forming monocultures of dense stands with high leaf area (Sala et al. 1996). The implications of *Tamarix* competition with native species for water and light are widespread. For example, riparian community structure and fluvial ecosystem processes are transformed following *Tamarix* introduction and establishment (Lovell et al. 2009), and water table depth can be altered by *Tamarix* monocultures (Johnson 1987).

Tamarix invasion is aided by both biotic and abiotic factors. The invasive nature of *Tamarix* has particularly benefited from anthropogenic disturbance to riparian ecosystems with dam construction and flow regulation. Dams disrupt natural flood regimes and alter fluvial processes (i.e., stream bank erosion and sediment deposition), which are integral to the establishment of many native riparian species (Fenner et al. 1985, Stromberg et al. 1991, Auble et al. 1994, Merritt and Cooper 2000, Mortenson and Weisberg 2010). With decreased flooding and low flows, waterways downstream of dams can experience higher salinity levels (Lee and Bell 1999, Havel et al. 2005), which aid in the

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establishment of salt-tolerant species (e.g., *Tamarix*) and inhibit the establishment of salt-sensitive native species (Siegle and Brock 1990, Busch and Smith 1995, Shafroth et al. 1995, Lovell et al. 2009). Moreover, native riparian species that are sensitive to water availability are negatively impacted by reduced water table height from river diversions and flow regulations (Smith et al. 1991, Stromberg et al. 1991).

Certain key biological characteristics have contributed to *Tamarix* invasibility. *Tamarix* disperses small, comose seeds in vast quantities throughout the growing season (Merkel and Hopkins 1957, Warren and Turner 1975). Continuous seed dispersal results in complete colonization of viable germination sites by *Tamarix* seedlings, and *Tamarix* seeds are able to germinate in soils with high salinity levels (Brotherson and Winkle 1986, Shafroth et al. 1995, Sala et al. 1996). Early growth strategies result in resource allocation to below-ground biomass, which augments the capacity of *Tamarix* to operate as a facultative phreatophyte (Brotherson and Winkle 1986, Busch et al. 1992). Overall, *Tamarix* growth and physiology allow it to potentially monopolize water resources in riparian systems (Sala et al. 1996).

As water availability has become an increasingly contentious issue in arid regions of the southwestern United States, concerns have been raised regarding *Tamarix* water use. Some studies have demonstrated that *Tamarix* water use is among the highest of any phreatophyte in the southwestern United States (Brotherson and Winkle 1986), including native riparian trees (Busch and Smith 1995). Other studies have challenged these earlier findings: depending on environmental conditions, *Tamarix* water use may vary, making its stomatal conductance of water vapor plastic (Cleverly et al. 2002, Owens and Moore 2007, Lovell et al. 2009, Nagler et al. 2013). Lovell et al. (2009) demonstrated that *Tamarix* has greater water-use plasticity than either *Populus* or *Salix*. In moist sites along the Arkansas River, Colorado, *Tamarix* had greater stomatal conductance than *Populus* or *Salix;* however, in drier sites, *Tamarix* had lower stomatal conductance than *Populus* or *Salix*. These data suggested that *Tamarix* might be able to maintain relatively higher carbon assimilation rates when water is plentiful and conserve more water under dry conditions than the other riparian species. Thus, *Tamarix* water use can depend on local environmental conditions.

When *Tamarix* does transpire less efficiently, high water use by *Tamarix* is largely due to the high leaf area index of *Tamarix* communities compared to other riparian populations (Sala et al. 1996). In areas of high density or leaf area, *Tamarix* has the ability to dry up springs, drain small ponds, and even desiccate perennial streams (Johnson 1987). Controlling *Tamarix* stands with high leaf area may conserve water, although the vegetation that replaces *Tamarix* will determine the magnitude of water conservation (Shafroth et al. 2005).

Varying management techniques have been utilized in attempts to control and manage *Tamarix*, with the hope of restoring riparian ecosystems and reducing water lost through evapotranspiration by *Tamarix*. Because *Tamarix* represents the sole genus from the family Tamaricaceae in North America, biological control with the tamarisk leaf beetle *Diorhabda* spp. (Coleoptera: Chrysomelidae) has been considered a viable management technique (Gaskin et al. 2004). The beetles feed by scraping away the cuticle to access mesophyll and vascular tissues within the leaves. To mitigate water loss from damaged leaf tissues, *Tamarix* abscises masticated leaves (Snyder et al. 2010). Because of reductions in photosynthate production, this leaf loss can lead to *Tamarix* mortality.

Since the original introductions occurred, the beetles have successfully spread from the original release sites and defoliated thousands of acres of *Tamarix* stands (Carruthers et al. 2008). The success of the tamarisk leaf beetle at dispersing to *Tamarix*-dominated riparian ecosystems has the potential to make this beetle species one of the most widespread biological control agents in recent history (Snyder et al. 2010). Although the tamarisk leaf beetle has succeeded at defoliating extensive *Tamarix* stands, the physiological impacts of the biological control agent on *Tamarix* are still widely unknown (Snyder et al. 2010) and could have ecosystem-wide consequences (Denslow and D'Antonio 2005).

Here, we examine the response of *Tamarix* to invasion by the tamarisk leaf beetle with respect to reproductive potential and a suite of functional traits. We measured stomatal

conductance, foliar chlorophyll and abscisic acid content, proportion of living stems, and flower production on individual tamarisk plants. Stomatal conductance is the relative rate that water vapor exits as carbon dioxide enters through leaf stomata (similar to transpiration rate). Foliar abscisic acid (ABA) is a phytohormone that causes stomatal closure, and when present in high concentrations can result in increased water-use efficiency and decreased water use (Davies et al. 1990, Heschel and Riginos 2005). Foliar chlorophyll content is indicative of photosynthetic potential and has been linked to stress tolerance in previous tamarisk work (Lovell et al. 2009). We asked the following research questions: How are *Tamarix* functional traits impacted by the biological control agent? How are *Tamarix* fecundity and survival strategies impacted by the biological control agent?

Fig. 1. Bankside *Tamarix* at Fountain Creek, Colorado.

METHODS

Study System

Riparian forests in Colorado have historically been composed of a diverse assemblage of forbs and graminoids interspersed among 2 dominant woody species in the Salicaceae family: the plains cottonwood (*Populus deltoides*) and the sandbar or coyote willow (*Salix exigua*) (Reichenbacher 1984). Since the early 1820s, up to 12 species from the genus *Tamarix* have been introduced into parts of the southwestern United States (Baum 1967, Crins 1989). Horticulturists performed the first introductions of *Tamarix* during the early nineteenth century from sources in Europe, Asia, and North Africa (Gaskin and Schaal 2002, Gaskin and Kazmer 2006). During the mid-1800s, *Tamarix* was planted by the Army Corps of Engineers along waterways as a bank stabilizer (Bean et al. 2013), and by the late nineteenth century, *Tamarix* species started to naturalize in the southwestern United States. The species present at our study sites in Colorado was *Tamarix ramosissima*. *Tamarix ramosissima* (hereafter *Tamarix*) is a deciduous shrub or small tree characterized by reddish stems, pale green foliage, and distinctive minute, pink flowers (Baum 1967; Fig. 1). It can be found in saline and xeric soils along riparian corridors in southeastern Colorado (Brotherson and Winkle 1986).

In an attempt to control *Tamarix* via foliar herbivory, management agencies have introduced the tamarisk leaf beetle (*Diorhabda* spp.) as a biological control agent in the southwestern United States (Hart et al. 2005). The most likely species found at our study sites in southeastern Colorado is *Diorhabda carinulata* (B. Drummond personal communication). *Diorhabda carinulata* (the northern tamarisk leaf beetle) is endemic to central Asia and is adapted to higher latitudes. Populations of this beetle have become widespread throughout Colorado, Montana, Wyoming, Utah, and Nevada. Following secure cage trials in 2001 (Dudley et al. 2001), experimental releases were carried out at 7 sites, including a site near Pueblo, Colorado. The beetles found at our Fountain Creek study sites during the 2013 field season most likely aggregated there after dispersing from a Pueblo, Colorado, introduction site.

Study Sites

Two sandbars, identified as northern and southern, along Fountain Creek were chosen as study areas near Fountain Creek Regional Park (38°42′07.51″N, 104°43′02.25″W). The Fountain Creek watershed drains approximately 930 square miles of southeastern Colorado and experiences seasonally varying flow regimes. These sandbars are relatively uniform in nature, although some distinct differences exist. The northern sandbar experiences higher light

levels compared to the southern sandbar. A mixed canopy of *Populus* and *Tamarix* provides some shade and decreases light penetration to the understory and soil surface at the southern site. Both sandbars have well-drained soils composed of sand and coarse sediment.

Experimental Design

TREE SELECTION AND SITE ENVIRONMENT MEASUREMENTS.—During the 2010 field season, 325 *Tamarix ramosissima* plants were tagged at the 2 sandbars along Fountain Creek. During the 2013 and 2014 field seasons, a total of 356 and 200 *Tamarix* individuals, respectively, were tagged at the same 2 sandbars. For all 3 years, the chosen *Tamarix* plants were haphazardly selected from the entire *Tamarix* population within 30 m of the stream bank at each site. The stem diameter (at 1 m from the soil surface) of selected plants ranged from 5 to 75 mm, with most plants having about a 25-mm stem diameter.

For each sandbar, light levels at the stem and at 1 m from the stem were collected in addition to soil moisture content. Approximately 100 light and moisture readings were taken across both sites each field season. A LightScout PAR (photosynthetically active irradiance) quantum meter (Spectrum Technologies, Aurora, IL) was used to measure light levels at each plant. Volumetric water content (VWC) at a 12-cm soil depth was measured using a TDR Moisture Meter (Campbell Scientific, Logan, UT) at each of the sandbars. Riverine data from the United States Geological Survey (USGS) for Fountain Creek, Colorado, were examined for flood frequency calculations. For functional trait measurements, we selected the most recently fully expanded leaves in order to control for leaf age.

FUNCTIONAL TRAIT MEASUREMENTS.—Stomatal conductance (g_{st}) measurements were collected to determine water-use rates for individual *Tamarix* leaves. A Steady State Diffusion Leaf Porometer (model SC-1, Decagon Devices, Pullman, WA) was used to collect all stomatal conductance measurements. Measurements were collected between 10:00 and 15:00 when light levels were greater than 900 μmol photons · $m^{-2}s^{-1}$. Terminal, fully expanded leaves on recent growth were selected for conductance measurements; 2 leaves were clamped with the sensor head on the abaxial surface. These data are estimates of leaf-level gas exchange for a single branch, rather than transpiration rates for an entire tamarisk plant. During the 2010 and 2013 field seasons, stomatal conductance was measured for 210 and 308 individuals, respectively, from late June until early July. During the 2014 field season, stomatal conductance was measured for 188 individuals during early July. Stomatal conductance data were adjusted for day and time-of-day effects with regression (see below).

Leaves were collected from randomly selected *Tamarix* individuals to quantify foliar chlorophyll content. For each individual, recent growth was sampled from the first main branch. Five to 6 terminal leaves were removed and placed directly on ice in a cooler. Samples were transferred to a dark, −20 °C freezer immediately upon arrival at the laboratory. During the 2010, 2013, and 2014 field seasons, 137, 129, and 46 individuals were sampled for chlorophyll analysis, respectively. Chlorophyll was extracted and quantified according to the protocol from Lovell et al. (2009). Leaf samples (300–350 mg) were pulverized in spectrophotometric-grade acetone with a Polytron tissue grinder (Polytron, Duluth, GA). To quantify foliar chlorophyll content, absorbance values were measured at 647 nm and 664 nm with a Genesys 20 Visible Spectrophotometer (Thermo Scientific, Waltham, MA).

Abscisic acid (ABA) was extracted and quantified from leaves collected at the northern site during the 2010 and 2013 field seasons, according to the protocol in Boggs et al. (2010). Samples collected in 2010 and 2013 were weighed and stored at −20 °C in the dark. From the stored leaf samples, 32 and 51 samples were chosen from 2010 and 2013, respectively. For both field seasons, about 300 mg of leaf tissue from each plant was lyophilized for 24 h. ABA was extracted from lyophilized leaf samples using a Polytron tissue grinder with minimal lighting. Leaf samples were ground in ABA extraction buffer containing methanol, butylated hydroxytoluene, and citric acid monohydrate (Bibee et al. 2011). Ground samples were stored in a −20 °C freezer until they were centrifuged; following centrifugation, supernatant was added to TBS (with $MgCl_2$) and vortexed.

ABA was quantified in leaf samples using ELISA (Agdia, Inc., Elkhart, IN). ABA standards (10^{-6} to 10^{-12} M; mixed isomers, Sigma-Aldrich) were prepared to generate a standard curve. For each microtiter plate, wells were loaded in the dark on ice, and substrate solution was added to each well. After incubation at 37 °C, we used an Optima Fluostar plate reader (BMG Labtech Inc., Cary, NC) to measure absorbance in each of the wells at 405 nm. Optical densities were recorded for the standards and samples to generate molar concentrations per milligram of leaf tissue (Boggs et al. 2010, Heschel et al. 2014).

To estimate tree health during the 2013 and 2014 field seasons, the numbers of living and dead stems were recorded for 308 and 168 individuals, respectively. From these data we calculated the proportion of living to dead stems. All stems were meticulously counted and recorded as either "dead" or "alive" on a given plant; "alive" stems had at least 50% of the stem covered in healthy, green foliage.

REPRODUCTION ESTIMATES.—For all 3 field seasons, inflorescences were counted and used to estimate total flower number for each individual. We define an inflorescence as a cluster of 6–8 racemes. We note that flower number does not equate with reproductive fitness; however, because of high selfing and outcrossing rates, many flowers in our populations do become fruits (Drummond and Heschel unpublished data).

For all tagged plants in a given year, we counted the number of inflorescences on each branch and then estimated the number of flowers for each of these inflorescences. To estimate mean flower number per inflorescence, we measured 10 racemes per individual. On the first major branch, 5 racemes from the terminal inflorescence were measured to the nearest millimeter. This measurement was repeated on the 2nd major branch for a total of 10 raceme lengths per plant. We used a regression model (see below) to estimate how many flowers each of these racemes contained, and calculated an average flower number per inflorescence for every plant. Finally, we totaled all flowers for each *Tamarix* plant by multiplying the average flower number per inflorescence by the total number of inflorescences per plant. This method allowed us to conservatively estimate the total number of flowers for each flowering *Tamarix* in our study. (To establish a relationship between raceme length and flower number, we first measured the length and total flower number for 5 racemes at the terminus of the lowest branch of 40 plants. Raceme length was then regressed against total flower number to generate a linear model [$R^2 = 0.72$].)

Data Analyses

All statistical analyses were performed with JMP version 7.0.2 (SAS Institute, Cary, NC). ANOVAs were used to test for functional trait and flower number differences between invasion periods; invasion period (treatment) and sandbar location (site) were considered fixed factors. Location (site) was included as a blocking factor to control for environmental effects on the north and south sandbars; a site-by-treatment interaction was not possible for ABA and chlorophyll concentration data due to sampling issues for the preinvasion year. Planned contrasts (*t* tests) were used to compare trait values between individual invasion time periods (statistical significance was determined at $P \leq 0.05$). Within a sampling year, stomatal conductance data were adjusted for effects of measurement day and time. Conductance values were regressed against measurement time, and residuals from this regression were added to the g_{st} grand mean (Bibee et al. 2011). For all statistical models, residual distributions were examined and the data were log-transformed where necessary. Log_{10} transformations were used to meet assumptions of normality.

Phenotypic "selection" analyses were used to examine the effects of functional traits on flower production for each invasion period (Heschel and Riginos 2005). Our conservative estimate of flower number is not equivalent to reproductive fitness in *Tamarix;* however, it does provide an estimate of reproductive potential/fecundity. Flower number data were relativized and trait data were standardized for each invasion period before linear regression analyses were performed. Relative flower number was determined by dividing total flower number by the grand mean for each invasion period. Traits were standardized to a mean of 0 with a standard deviation of 1. Differential analysis was a simple linear regression of the standardized trait on relative flower number. Gradient analysis was a multiple regression of all standardized traits on relative flower number.
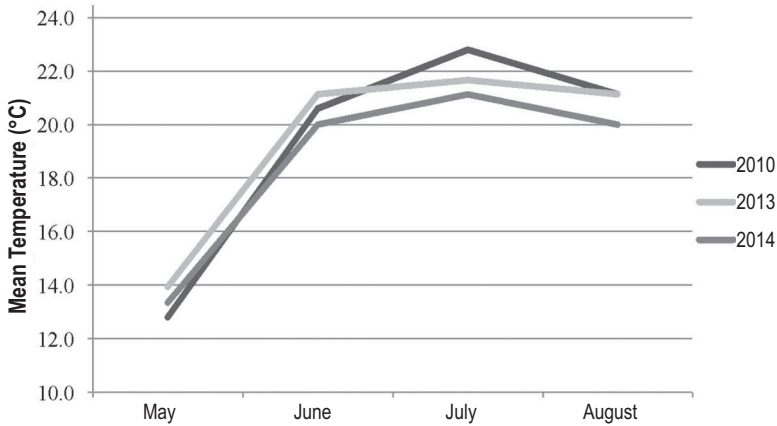
Fig. 2. Mean monthly temperature in Fountain, Colorado, during the 2010 (pre–beetle invasion), 2013 (during–beetle invasion), and 2014 (post–beetle invasion) field seasons.
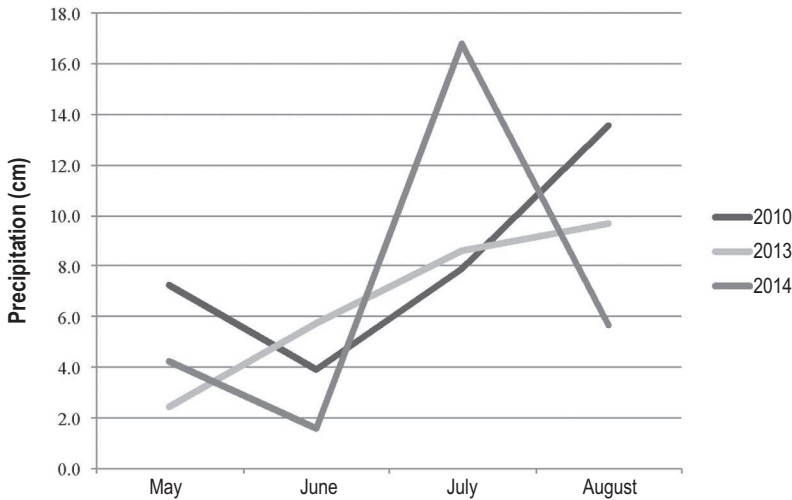

Fig. 3. Total monthly precipitation in Fountain, Colorado, during the 2010 (pre–beetle invasion), 2013 (during–beetle invasion), and 2014 (post–beetle invasion) field seasons.

RESULTS

Site Environmental Conditions

FLOOD FREQUENCY AND DISCHARGE.— United States Geological Survey (USGS) data for Fountain Creek, Colorado, collected by station number 07106000 demonstrate differences in mean gage height between invasion periods. Gage heights were averaged from June to August for each invasion time period. The post–beetle invasion time period had the highest gage height ($\overline{x} = 1.46$ m), whereas during the beetle invasion, gage height was

the lowest ($\overline{x} = 0.973$ m). The post–beetle invasion period was characterized by an increase in flood events leading to high channel height and stream flow. In 2010, the mean monthly discharge of Fountain Creek from May through August ranged from 82.5 ft$^3 \cdot$ s^{-1} to 154.5 ft$^3 \cdot$ s^{-1}. In the 2013 field season, the mean monthly discharge ranged from 25.7 ft$^3 \cdot$ s^{-1} to 141.1 ft$^3 \cdot$ s^{-1}. In 2014, the mean monthly discharge ranged from 57.3 ft$^3 \cdot$ s^{-1} to 147.4 ft$^3 \cdot$ s^{-1}.

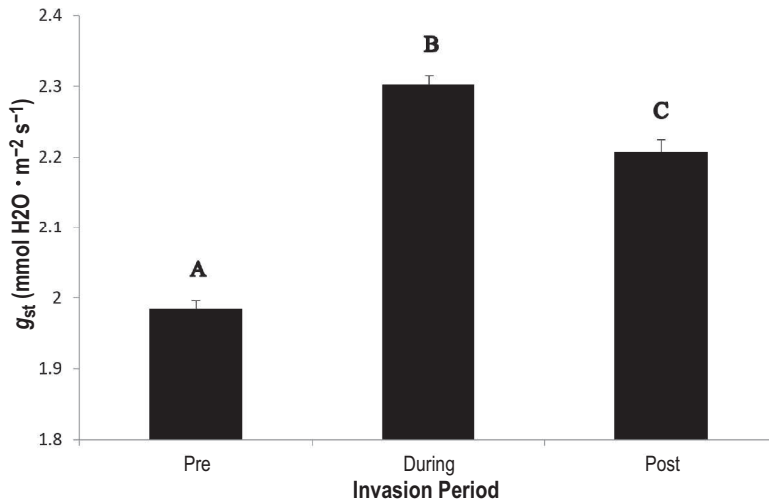TEMPERATURE, PRECIPITATION, AND LIGHT LEVELS.—Temperature and precipitation data

Fig. 4. Mean stomatal conductance measurements (±1 SE) for *Tamarix* individuals during the pre–, during–, and post–beetle invasion time periods. Conductance values were adjusted for measurement time. Shared letters indicate a lack of statistical significance with planned contrasts.

TABLE 1. ANOVA results for invasion period (pre–, during–, and post–beetle herbivory) and site (south and north) on physiological traits. ABA = foliar abscisic acid concentration per milligram fresh leaf mass; g_{st} = stomatal conductance. *F* values are reported from effect tests. NA = not applicable.

	Log g_{st}	ABA	Chlorophyll	Total flower number	Proportion alive
Invasion period	185.35***	3.499[+]	25.4445***	8.8710***	36.6887***
Site	0.1488	NA	2.8953[+]	0.7820	1.5019
Invasion by site	0.0509	NA	NA	0.7287	18.8251*

+ *P* < 0.1   ** *P* < 0.01
* *P* < 0.05   *** *P* < 0.001

(Figs. 2, 3) were collected from a weather station near Butts Army Airfield at Fort Carson, Colorado, which is within 16 km (10 miles) of our study sites. Mean monthly temperatures for June during 2010, 2013, and 2014 ranged from 19.4 to 21.1 °C. In July over the same time period, the average monthly temperature ranged from 21.1 to 22.8 °C. In 2010 and 2013, precipitation accumulation from May through August ranged from 3.9 to 13.6 cm and 2.4 to 9.7 cm, respectively. In 2014, precipitation ranged from 1.5 cm to 16.8 cm for this same time period. For June and early July, the precipitation ranged from about 4 cm to 8 cm across all 3 years, with 2014 being the wettest year. Volumetric water content measurements ranged from 2% to 11% across both sandbars during all 3 growing seasons. For the northern sandbar, light levels ranged from about 1200 to 1900 μmol photons · m^{-2} s^{-1}. For the southern sandbar, light levels ranged from 400 to 1820 μmol photons · m^{-2} s^{-1}.

Tamarix Water Relations

Invasion by the tamarisk leaf beetle had a significant effect on leaf-level stomatal conductance (Table 1); moreover, contrasts indicated that stomatal conductance values were significantly different for each invasion period (Fig. 4). Conductance was lowest during the pre–beetle invasion time period, highest during the beetle invasion, and intermediate during the post–beetle invasion period (Fig. 4). Invasion period had a marginally significant effect on foliar abscisic acid content (Table 1). Foliar abscisic acid content was lower during the pre–beetle invasion period than during the beetle invasion, but these differences were small (mean molar ABA per mg leaf weight: 2010
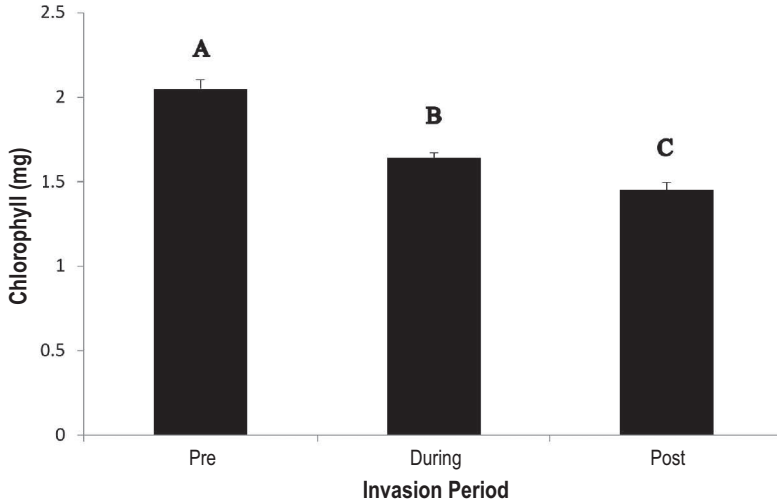
Fig. 5. Mean foliar chlorophyll content (±1 SE) of *Tamarix* for pre– and during–beetle invasion time periods. Shared letters indicate a lack of statistical significance with planned contrasts.
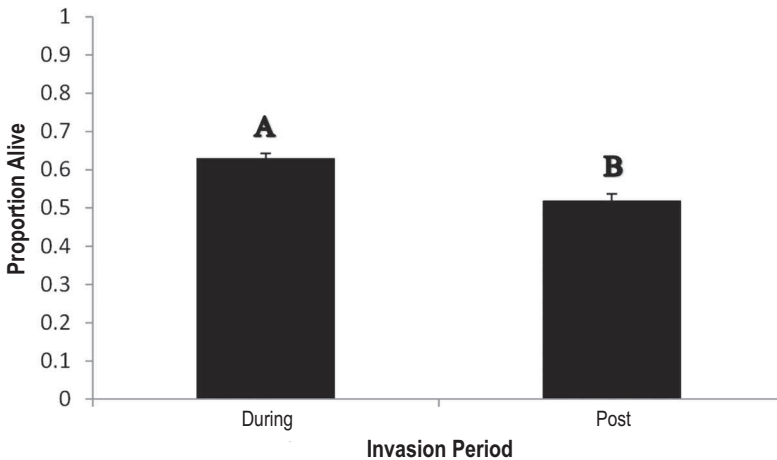

Fig. 6. Mean proportion of living stems (±1 SE) on *Tamarix* for during– and post–beetle invasion time periods. Shared letters indicate a lack of statistical significance with planned contrasts.

[pre–beetle invasion] = 2.41×10^{-10} [SE 2.40×10^{-10}], 2013 [during] = 8.17×10^{-10} [SE 1.91×10^{-10}]).

Foliar Chlorophyll Content

Invasion period had a significant effect on foliar chlorophyll content (Table 1). Chlorophyll content declined across the invasion periods; foliar chlorophyll content was highest before the beetle invasion, decreased during the beetle invasion, and decreased again following the beetle invasion (Fig. 5). These changes in leaf chlorophyll content should translate into differences in photosynthetic potential. There-fore, decreases in foliar chlorophyll might result in less biomass accumulation and a potential reduction in reproductive effort.

Proportion of Stems Alive

There was a significant effect of invasion period on the proportion of living stems (Table 1). During the beetle invasion there was a higher proportion of living stems than during the post–beetle invasion period (Fig. 6);
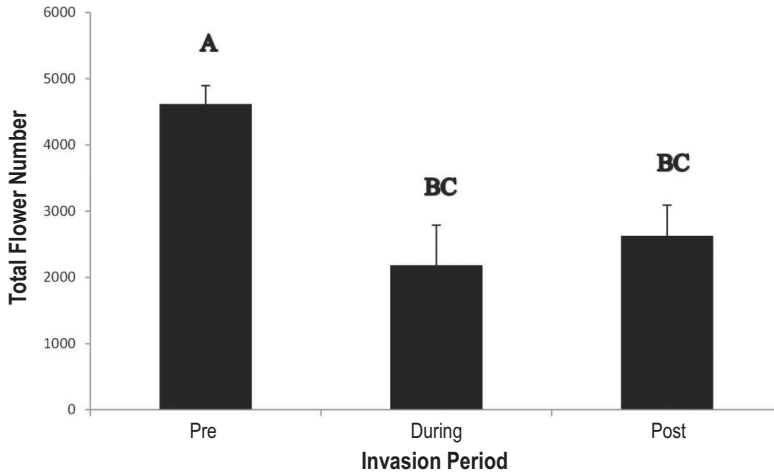
Fig. 7. Mean total flower numbers (±1 SE) of *Tamarix* plants for the pre–, during–, and post–beetle invasion time periods. Shared letters indicate a lack of statistical significance with planned contrasts.

TABLE 2. "Selection" analyses for functional traits for pre–, during–, and post–beetle invasion time periods (i.e., associations between standardized traits and relative flower number). Differential "selection" coefficients (S) were slope terms from linear regressions, and gradient "selection" coefficients (β) were slope terms from multiple regressions. NA = not applicable (data absent).

	Pre-beetle		During-beetle		Post-beetle	
	S	β	S	β	S	β
g_{st}	0.14389+	0.75597+	−0.17723	−0.22507	0.018181	0.011658
Chlorophyll	0.061062	−0.59868	0.442389+	0.333622	0.285468*	0.300575+
ABA	−0.42017*	−0.43277+	−1.12939	−1.76216	NA	NA

+ $P < 0.1$
* $P < 0.05$

also, the southern site experienced stronger herbivory than the northern site (site-by-invasion interaction, Table 1). The pre–beetle invasion period was characterized by trees which leafed out early in the season and had a high proportion of alive stems (observational and photographic data, S. Heschel). This reduction in total leaf area for individual tamarisk plants reduced photosynthetic area but also reduced the total number of stomata for a given plant.

Tamarix Flower Production and "Selection"

The tamarisk leaf beetle had a significant effect on *Tamarix* flower production (Table 1). Estimated total flower number was highest during the pre–beetle invasion period, lowest during the beetle invasion, and intermediate during the post–beetle invasion period (Fig. 7). It was assumed that total flower number is a rough proxy for *Tamarix* fecundity, so "selection" analyses were conducted on the functional data (Table 2). During the pre–beetle invasion period, individuals with higher stomatal conductance and lower ABA content produced more flowers. Increased conductance and decreased ABA may have helped to reduce leaf temperatures while maintaining photosynthesis with gas exchange (Heschel and Hausmann 2001). During the beetle invasion time period, individuals with higher foliar chlorophyll content produced more flowers, but ABA content did not significantly predict flower number (Table 2). During the post–beetle invasion period, individuals with high foliar chlorophyll content had greater flower production (Table 2). Stomatal conductance was positively associated with flower number during this time period, but not significantly so (Table 2).

DISCUSSION

Invasion by the tamarisk leaf beetle during the 2013 field season impacted both *Tamarix* functional traits and flower production. During invasion by the biological control agent, leaf-level stomatal conductance and ABA content increased, whereas foliar chlorophyll content and total flower number decreased relative to preinvasion levels. Following the beetle invasion, leaf-level stomatal conductance was still relatively high, whereas foliar chlorophyll content and the proportion of living stems significantly decreased relative to levels during the beetle invasion; however, flower number did not significantly change after the invasion. Our results suggest that herbivory by the leaf beetle significantly impacts *Tamarix* populations during both a defoliation event and the subsequent growing season. Furthermore, trait associations with flower number seem to be impacted by the biological control agent.

Biological Control and *Tamarix* Water Use

The beetle had a significant impact on leaf-level stomatal conductance at our study sites. Our results provide an interesting addition to previously reported findings on *Tamarix* water use in response to the biological control agent. Snyder et al. (2010) demonstrated in a controlled greenhouse environment that stomatal conductance increased in plants with beetles present. Moreover, their data indicated that beetle herbivory decreased photosynthesis and produced leaves that were unable to effectively regulate water loss. In a field setting, Pattison et al. (2011) also found that beetle defoliation inhibited the ability of *Tamarix* to regulate water use (in part because of changes in biomass allocation). Thus, both of these data sets indicated that beetle herbivory might make *Tamarix* less drought tolerant. Our field results of increased stomatal conductance during and after beetle invasion corroborate these findings. However, this increased water use by *Tamarix* plants might not be as dramatic as our data suggest due to the leaf loss caused by beetle herbivory; the conductance data presented here are at the individual leaf level. Also, the increased water use during the year following beetle herbivory may in part be due to a slight increase in precipitation during 2014.

In addition to impacts on water use, herbivory by the tamarisk leaf beetle results in a significant loss in photosynthetic area (Fig. 6). To compensate for this loss of photosynthetic area, *Tamarix* leaves might plastically respond to beetle herbivory by increasing chlorophyll production to enhance photosynthetic potential in remaining foliar tissues. *Tamarix* plants also might produce more leaves in order to increase photosynthetic area. However, both of these defoliation response strategies require a costly energetic input. In our *Tamarix* population, plants that increased photosynthetic potential produced more flowers (Table 2), but beetle herbivory stress decreased chlorophyll content and total leaf area during and after the invasion time periods. Therefore, our results indicate that photosynthetic potential may be negatively impacted by the leaf beetle control agent.

A tradeoff seems to exist here between drought response and photosynthetic rate; individuals experiencing diminished photosynthetic potential must increase gas exchange with high g_{st} to maintain carbon fixation rates. *Tamarix* leaves at our study sites responded to herbivory by decreasing chlorophyll content. During the invasion, this reduction seemed to require *Tamarix* leaves to open stomata for extended periods of time (Fig. 4) to allow sufficient gas exchange to maintain photosynthesis (Larcher 2003). Consequently, transpiration and water loss would have increased as stomata remained open, and beetle herbivory might have contributed to further water loss from masticated tissues. Thus herbivory by the leaf beetle seems to stress the tradeoff between drought response and photosynthetic rate. Although it should be noted that at the whole-plant level, a loss of photosynthetic area and total stomatal density with herbivory would help to ameliorate this tradeoff.

Abscisic acid may be functioning to promote this physiological tradeoff. ABA increased slightly during the invasion in response to water stress and/or to facilitate abscission of leaves damaged via beetle herbivory. Stomatal conductance also increased during the invasion. Plants experiencing increased ABA content and increased stomatal conductance can also exhibit low ABA sensitivity (cf. Heschel and Hausmann 2001, Heschel et al. 2014); such low ABA sensitivity may decrease

conductance response time. The rapid closure of stomata during drought can help to promote stress tolerance. Thus, these individuals may be compromising drought response by increasing water use with low ABA sensitivity in order to maintain carbon fixation rates. Under these conditions, water use becomes less efficient and *Tamarix* may "steal" water from neighboring individuals, potentially impacting water accessibility for native species.

Biological Control and *Tamarix* Reproduction

Because decreased photosynthetic potential can impair photosynthate production and exacerbate fundamental tradeoffs in *Tamarix,* some physiological demands may suffer. Our results indicate that the leaf beetle significantly decreases flower production both during and after the invasion relative to preinvasion flower numbers. Thus, intense defoliation during one growing season seems to impact *Tamarix* growth during the following growing season as well. This impact might be due to decreased resource allocation to belowground biomass, possibly in favor of generating reproductive structures. Intense foliar herbivory by the tamarisk leaf beetle might diminish the ability of *Tamarix* to accumulate nonstructural carbohydrate reserves in belowground biomass (Dudley and Bean 2011). During the beginning of the growing season, foliar growth (e.g., bud break and leaf out) relies on stored nonstructural carbohydrate reserves. Without a sufficient supply of stored resources from the previous growing season, *Tamarix* growth can be inhibited (Pattison et al. 2011), and such reductions in growth might decrease the ability of *Tamarix* to access water resources. Moreover, *Tamarix* flowering phenology may be delayed, which might allow native species that established earlier in the growing season to shade *Tamarix* and further decrease growth potential (Sher et al. 2002, Beauchamp and Stromberg 2007).

Conservation Implications

Although herbivory resulted in decreased chlorophyll, increased leaf-level conductance, and overall reduced flower counts, more fecund plants were able to maintain photosynthetic potential with relatively high foliar chlorophyll content, while mitigating additional water loss with low stomatal conductance (Table 2). That is, we detected a significant correlation between fecundity and leaf chlorophyll content. Given the tamarisk leaf beetle's impact on flower production and functional traits, the biological control agent appears to exert a significant selective pressure on *Tamarix*. During and after beetle invasion, plants with relatively low foliar chlorophyll content in their leaves might increase their stomatal conductance in an attempt to maintain relatively higher carbon fixation rates and increase flower production/fecundity. *Tamarix* populations that have relatively lower chlorophyll might therefore impact the local water table, and high transpiration rates by dense stands of *Tamarix* might severely impact water availability for native species (Johnson 1987). Thus, despite the success of the tamarisk leaf beetle at defoliating *Tamarix* stands, a reexamination of the use of biocontrol might be necessary in parts of the southwestern United States where water is particularly scarce (Thomas and Reid 2007).

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