

# Defoliation by introduced *Diorhabda elongata* leaf beetles (Coleoptera: Chrysomelidae) reduces carbohydrate reserves and regrowth of *Tamarix* (Tamaricaceae)

Jeremy L. Hudgeons<sup>a,\*</sup>, Allen E. Knutson<sup>a</sup>, Kevin M. Heinz<sup>b</sup>, C. Jack DeLoach<sup>c</sup>,  
Tom L. Dudley<sup>d</sup>, Robert R. Pattison<sup>e</sup>, Jim R. Kiniry<sup>c</sup>

<sup>a</sup> Texas A&M Research and Extension Center, 17360 Coit Road, Dallas, TX 75252, USA

<sup>b</sup> Department of Entomology, Texas A&M University, College Station, TX 77843-2475, USA

<sup>c</sup> US Department of Agriculture, Agricultural Research Service, Grassland Soil and Water Research Laboratory, 808 E. Blackland Road, Temple, TX 76502, USA

<sup>d</sup> Marine Science Institute, UC Santa Barbara, Santa Barbara, CA 93106-6150, USA

<sup>e</sup> Environmental and Natural Resource Institute, University of Alaska, Anchorage, AK 99501, USA

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## Abstract

*Diorhabda elongata* (Brullé) *sensu lato* leaf beetles have been released in the United States for the classical biological control of invasive *Tamarix* L. species, which are exotic trees that are causing deterioration of riparian ecosystems in western North America. The impact of *D. elongata* defoliation on *Tamarix* nonstructural carbohydrates (NCHOs) was measured in both manipulative field cage, and non-manipulative natural experiments. Additionally, spring above-ground growth was measured following beetle defoliation in manipulative field cage experiments in Texas. There was no significant difference in the proportional change in NCHOs between beetle-damaged and undamaged control-treatment trees in the manipulative field cage experiment. However, spring above-ground regrowth was reduced by 35% on trees which experienced beetle defoliation the previous fall. In the natural experiment, root crown tissue was sampled in 2005 and 2006 from stands near Lovelock, Nevada in which trees had experienced 0–4 years of beetle defoliation. In 2005, mean NCHO concentrations were statistically different between tree stands and ranged from  $9.0 \pm 0.8\%$  (mean  $\pm$  SE) in trees that had not been defoliated to  $3.2 \pm 0.4\%$ ,  $2.1 \pm 0.4\%$  and  $2.3 \pm 0.4\%$  in trees in stands that had been defoliated for 1, 2 and 3 successive years, respectively. In 2006, NCHO concentrations were again statistically different between stands and ranged from  $13.6 \pm 0.9\%$  in trees that had not been defoliated to  $7.6 \pm 0.8\%$ ,  $2.3 \pm 0.4\%$ ,  $1.5 \pm 0.3\%$  and  $1.7 \pm 0.4\%$  in trees in stands that had been defoliated for 1, 2, 3 and 4 years, respectively. These results indicate that *D. elongata* herbivory reduces nonstructural carbohydrates and inhibits regrowth which may lead to reduced survival and reproduction of *Tamarix*.

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## 1. Introduction

One of the most serious invasions of a noxious weed within the United States involves species of the exotic genus *Tamarix* L. (Tamaricales: Tamaricaceae) (Robinson, 1965; Stein and Flack, 1996; DeLoach et al., 2000).

*Tamarix* species are woody perennial trees or multi-stemmed shrubs native to arid riparian habitats of Eurasia and Africa (Baum, 1978). Between 8 and 12 species of *Tamarix* have been introduced into North America since the early 1820s (Baum, 1967; Crins, 1989) for planting as ornamentals, for windbreaks, to provide shade, and to stabilize eroding stream banks (Neill, 1985). All introduced species except *Tamarix aphylla* (L.) Karsten are deciduous and commonly referred to as saltcedar or tamarisk. By the

\* Corresponding author. Fax: +1 972 952 9632.

E-mail address: [jhudgeons@tamu.edu](mailto:jhudgeons@tamu.edu) (J.L. Hudgeons).

1920s *Tamarix* species had escaped cultivation and were becoming serious threats to arid riparian ecosystems in North America (Brotherson and Field, 1987). The invasive taxa in the United States are *Tamarix ramosissima* Ledeb., *Tamarix chinensis* Lour., *Tamarix parviflora* DC, *Tamarix gallica* L. and hybrids of these (Gaskin and Schaal, 2002). These species have invaded over 500,000 hectares of riparian habitat in the western United States (Robinson, 1965). The *Tamarix* invasion is often reported to have negative ecological effects such as displacement of native vegetation (Brotherson and Field, 1987), reduction in faunal diversity (Anderson et al., 1977; Kerpez and Smith, 1987; DeLoach et al., 2000; Knutson et al., 2003; Shafroth et al., 2005), increased sedimentation and bank aggradation (Brotherson and Field, 1987), increased channelization (Blackburn et al., 1982) and a lowering of water tables resulting from high evapotranspiration rates (Smith et al., 1998; Nagler et al., 2005).

### 1.1. Biological control program

To help combat the *Tamarix* invasion, the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) initiated a biological control research program in the late 1960s which resulted in the release of *Diorhabda elongata* (Brullé) *sensu lato* leaf beetles (Coleoptera: Chrysomelidae) (DeLoach et al., 2003). The adult and all three larval instars feed on the foliage of *Tamarix*. Two and sometimes three generations can be produced in North America, prior to entrance of adults into reproductive diapause in response to shortened daylength (Bean et al., 2007). The adult stage overwinters in the leaf litter and becomes active around spring budbreak. Females oviposit 128–280 eggs in masses (3–8 eggs per mass) on the host foliage (Lewis et al., 2003b; Milbrath et al., in press). In its native range, *D. elongata* herbivory can cause heavy to complete defoliation of *Tamarix* (DeLoach et al., 2003).

### 1.2. Host impact and the effects of defoliation

For *D. elongata* to be a successful biological control agent, its defoliation must have a significant impact on *Tamarix* growth and survival. Leaf-chewing insects have been important to successful biological control programs because they remove photosynthetic tissue, which reduces the ability of plants to maintain growth and vigor. However, the consequences of *D. elongata* herbivory on *Tamarix* growth and vigor are largely unknown. While *D. elongata* can completely defoliate *Tamarix* trees, plants can re-foliate within weeks of defoliation. To recover from defoliation, plants need adequate carbohydrate reserves to regenerate new leaf tissue (Chapin et al., 1990; Loescher et al., 1990). Stored carbohydrates in woody plants serve important roles in metabolism, growth, development of cold hardiness, defense and survival (Kozłowski, 1992). Thus a reduction in carbohydrate reserves in *Tamarix* by *D. elongata* defoliation could lower plant growth and vigor.

The removal of photosynthetic tissue by defoliation has been documented to lower carbohydrate storage reserves in some plants. Reduction in carbohydrate reserves following artificial defoliation of eight salt-desert shrub species was the result of continued respiration, reduction in photosynthesis and the use of reserves in producing regrowth (Trlica and Cook, 1971). Reserve carbohydrates were reduced while supporting new leaf growth following artificial defoliation of honey mesquite, *Prosopis glandulosa* Torr. (Cralle and Bovey, 1996). Contrary to these results, the transient effects on carbohydrate reserves and the rapid recovery of growth revealed the tolerance of healthy stands of hybrid poplar, *Populus* × *canadensis* cv Eugeneii, to outbreaks of the defoliating gypsy moth, *Lymantria dispar* L. (Kosola et al., 2001).

Woody plants accumulate and store carbohydrate reserves during periods when supply exceeds demands for maintenance and growth (Oliveira and Priestley, 1988; Kozłowski et al., 1991). Concentrations of nonstructural carbohydrates have been used to measure metabolic reserves in many plants including salt-desert shrubs (Trlica and Cook, 1971); sugar maple, *Acer saccharum* Marsh (Renaud and Mauffette, 1991); poplar, *Populus* spp. (Kosola et al., 2001); honey mesquite, *P. glandulosa* Torr. (Cralle and Bovey, 1996); Chinese tallow, *Sapium sebiferum* L. (Conway et al., 1999); and *Tamarix* (Sosebee, 2004). Nonstructural carbohydrates (NCHOs) are accumulated and stored resources which can be remobilized to support biosynthesis for growth or other plant functions. Starch, sucrose and reducing sugars comprise the NCHOs, whereas cellulose, lignin and hemicellulose are primarily structural in nature and not available as reserves (Weinmann, 1947; Loescher et al., 1990). Based on the plants' reliance on reserve carbohydrates following the stress of defoliation, we predict that defoliation by *D. elongata* will reduce *Tamarix* nonstructural carbohydrates. Additionally, we predict that insect defoliation will result in a reduction in spring above-ground regrowth which may ultimately lead to tree death.

The objectives of this study were to determine the impact of *D. elongata* defoliation on (1) the nonstructural carbohydrate concentrations of *Tamarix* root crowns in both manipulative field cage and non-manipulative natural experiments and (2) the spring above-ground regrowth in field cage experiments. These results will help determine the potential impact of *D. elongata* as a biological control agent of *Tamarix* species.

## 2. Materials and methods

### 2.1. Insect species

Insects used in the field cage experiment at Lake Thomas, Texas in 2004 and 2005 originated from beetles collected on *Tamarix* species 3 km west of Sfakaki, Crete, Greece (latitude 35.38°N, longitude 24.6°E, elevation 7 m) in April 2002. The insects released into the field near

Lovelock, Nevada in 2001 originated from beetles collected from *Tamarix* species 7 km west of Fukang, China (latitude 44.16°N, longitude 87.98°E, elevation 567 m) in July 1999. Beetles collected from Crete were identified as *Diorhabda elongata elongata* (Brullé) and from Fukang as *Diorhabda elongata deserticola* Chen by I.K. Lopatin (Professor, Byelorussian University, Minsk, Belarus).

All imported beetles came through the USDA-ARS Exotic and Invasive Weed Research Unit quarantine facility at Albany, California where parasites, predators and other organisms were removed. Beetles (eggs and adults) were subsequently sent to the USDA-ARS Arthropod Containment Facility at Temple, Texas and maintained on cultivated *Tamarix* in laboratory and field cages where details regarding beetle biology (Lewis et al., 2003b) and host range (DeLoach et al., 2003; Lewis et al., 2003a; Milbrath and DeLoach, 2006a,b) were examined. Voucher specimens of *D. elongata elongata* used in the field cage study and *D. elongata deserticola* from the Lovelock release site were deposited to the Texas A&M University Insect Collection, College Station, Texas (under Lot Nos. 663 and 665, respectively).

### 2.2. Manipulative field cage experiment, Lake Thomas, Texas

The effect of *D. elongata elongata* defoliation on *Tamarix* nonstructural carbohydrates (NCHOs) and regrowth under field cage conditions was determined by caging beetles on individual trees in a *Tamarix* stand on the western end of Lake J.B. Thomas (latitude 32.61°N, longitude 101.24°W, elevation 675 m) in Borden County, Texas (Fig. 1A). The field cage experiment was conducted during the 2004 growing season and replicated during the 2005 growing season. A randomized stratified block design was used with an individual *Tamarix* tree as the experimental unit. Only trees which could fit within a field cage without modifications were used. Cages were a square 3.3 m on the sides and 2 m in height. Each cage was covered with 20 × 20 mesh, Lumite fabric (Synthetic Industries, Gainesville, GA, USA). Cages facilitated the replication of beetle treatments by confining beetles and excluding insect predators which may inhibit *D. elongata elongata* population increases. Tree volume estimates were made from measures of canopy diameter and total height [volume =  $(\pi) \times (\text{diameter}/2)^2 \times (\text{height})$ ]. Trees were stratified into blocks based on estimated volume, and one tree from each block was randomly assigned one of the following three treatments: caged beetle treatment (20 mating pairs of *D. elongata elongata* per tree); cage controls (cages with no beetles added); and no-cage, no-beetle controls. The no-cage treatment controlled for experimental artifacts due to cage effects. The 2004 study included six replicates for each treatment. Typical tree height and width (at drip line) were  $1.9 \pm 0.04$  m (mean ± SE) and  $2.0 \pm 0.1$  m, respectively. The 2005 study used a whole new set of trees and included 10 replicates for each treatment. Typical tree height and

width (at drip line) were  $1.6 \pm 0.04$  m (mean ± SE) and  $1.0 \pm 0.04$  m, respectively.

Prior to inoculation with beetles, core samples were taken from the tree root crowns (July 2004 and 2005) and analyzed for nonstructural carbohydrates as described below. Following defoliation, cages and beetles were removed from the trees and root crowns were re-sampled (September 2004 and December 2005). To measure the regrowth potential of tree root crowns following defoliation, all above-ground tissue from the experimental trees was removed while the trees were dormant (December 2004 and 2005). Spring regrowth was quantified by removing, oven drying and weighing all new shoot and leaf biomass the following spring (May 2005 and 2006).

### 2.3. Non-manipulative natural experiment, Lovelock, Nevada

Heavy snowfall during 1982–1983 resulted in flooding along the Humboldt River in Nevada. As the waters receded from the terminal basin, *Tamarix* invaded the Humboldt sink. Natural Resource Conservation Service (NRCS) officials estimate more than 5000 hectares or 60% of the sink area is exclusively *Tamarix* canopy (Stevenson, 1996). In the summer of 2001, approximately 1650 *D. elongata deserticola* adults were released into a monotypic stand of *Tamarix* in the lower Humboldt sink near Lovelock, Nevada (Fig. 1B). The release has resulted in temporally different and spatially segregated defoliation of *Tamarix* at the release site. By the end of summer 2002, the beetles had multiplied, dispersed and defoliated trees occupying an estimated 2 hectares surrounding the release origin. At the end of the 2003 and 2004 season, the beetles had defoliated trees occupying an estimated 200 and 1800 ha surrounding the release origin, respectively (Geraci, 2006).

By the end of 2004, four areas within the terminal sink of the Humboldt River could be distinguished by the number of consecutive years the trees had experienced defoliation by *D. elongata deserticola*. In this study, each spatially segregated area of defoliation was considered a separate treatment: treatment ‘three’ includes trees from the 2 ha which had experienced beetle defoliation for 3 consecutive years; trees in treatments ‘two’ and ‘one’ had experienced 2 and 1 years of defoliation, respectively. Trees in treatment ‘zero’ had experienced little to no defoliation by the end of the 2004 season. Prior to spring bud break (March 2005), the root crowns of 30 trees were sampled from treatments two, one and zero, and 15 trees were sampled from treatment three using the method described below. Typical tree height and width (at drip line) were  $3.6 \pm 0.06$  m (mean ± SE) and  $2.8 \pm 0.01$  m, respectively.

By the end of the 2005 season, the beetles had defoliated trees occupying an estimated 8100 ha (Geraci, 2006). As a result, all previously sampled treatment trees had experienced one additional year of beetle defoliation including

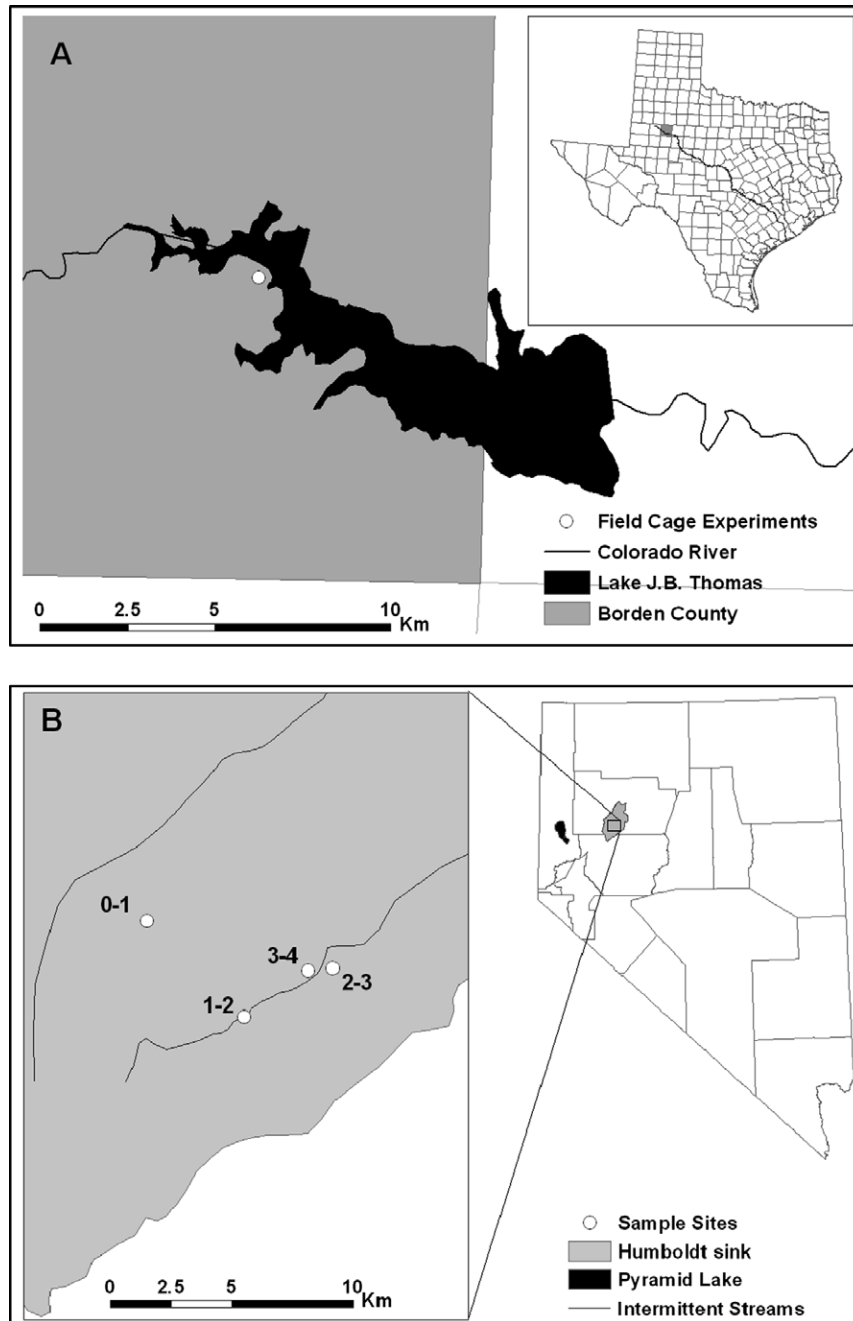


Fig. 1. Field cage experiment site near Lake Thomas in Texas (A) and natural experiment site in Nevada. (B) Numbers next to Nevada sample sites indicate treatment: number of years of tree defoliation by *D. elongata deserticola* in 2005 and 2006. Trees at Pyramid Lake were sampled in 2006 to serve as 'zero' years of defoliation treatment.

trees from treatment 'zero'. In early April 2006, prior to budbreak, the same trees sampled in 2005 were re-sampled and considered as the 2005 treatment plus one (e.g. trees from treatment 'three' in 2005 were considered as treatment 'four' in 2006). Few trees could be found in the lower Humboldt sink which had not been defoliated by *D. elongata deserticola* after the 2005 season. As a consequence, 15 trees from Pyramid Lake (another terminal basin approximately 75 km E–SE from the Humboldt sink sites) were sampled to serve as the 'zero' treatment in 2006 (Fig. 1B).

#### 2.4. Tissue collection and enzymatic analyses

Tissue for NCHO analysis was taken from the root crown of the trees. The root crown is defined as the tissue between the branching stems and the roots of a plant and is located at or just below the soil surface. The root crown has been noted as a carbohydrate storage organ in woody plants (Trlica and Cook, 1971) including *Tamarix* (Sosebee, 2004). Root crown tissue was removed using an 18 V cordless drill and a 20-mm ( $\frac{3}{4}$ ) wood boring bit. The bark

layer was removed from the point of sampling using the boring bit, the crown was bored 10 mm deep, and wood shavings were collected in aluminum foil placed under the boring bit. Holes were plugged with bees wax to prevent infections from entering the tree wound. Tissue samples were stored on dry ice in the field. Upon return to the laboratory, the samples were heated to 100 °C for 90 min to halt any innate enzymatic activity and then dried at 65 °C for 72 h to remove all moisture. After drying, each sample was ground separately using a Wiley mill fitted with a 40 mesh (0.5 mm) screen. Samples were stored in air tight vials in the dark and at room temperature until carbohydrate analysis.

Root crown samples were analyzed for sucrose, glucose, fructose and starch concentrations using the enzymatic method (Smith et al., 1964; McBee et al., 1983; Kiniry, 1993) with some modifications. A 0.25-g sample of dry material was measured into a disposable 50 ml centrifuge tube and extracted three times with 95% ethanol at 80 °C for 30 min. The supernatants from each sample extraction were decanted and combined in a clean 50 ml centrifuge tube. The resulting ethanol extractions contained the water soluble sugars sucrose, glucose and fructose. Pellet residues were saved for subsequent starch analysis. A colorimetric enzyme kit (#E0716260, R-Biopharm Inc., Marshall, MI, USA) and spectrophotometer (Spectronic 601, Milton-Roy, Ivyland, PA) were employed to determine sucrose, glucose and fructose concentrations from 200 µl aliquots of the ethanol extractions. The residue pellets remaining after the aqueous extraction were resuspended in 15 ml of distilled water and heated at 95 °C for 30 min and then cooled on ice for 10 min. After cooling, 10 ml of 100 mM sodium acetate buffer (pH 4.5) and 20 µl of amyloglucosidase (11,500 U/ml, A-3042, Sigma–Aldrich, St. Louis, MO, USA) were added to each sample, and the samples were digested for 24 h at 55 °C. Amyloglucosidase hydrolyzes starch to quantitative yields of glucose. From the digested starch solution, 100 µl samples were analyzed for glucose, and starch concentrations were calculated from the glucose yields. Water soluble sugar (WSS) and starch concentrations were calculated as a percentage of sample dry weight, and tree NCHO concentration was reported as the sum of the WSS and starch concentrations.

### 2.5. Statistical analyses

For the manipulative field cage experiment, proportional changes in NCHOs were calculated as  $([\text{post-treatment NCHOs}] - [\text{pre-treatment NCHOs}]) / [\text{pre-treatment NCHOs}]$ . Data were transformed in cases where ANOVA assumptions for normality and equality of variance could not be verified from results generated by the Shapiro–Wilk test for normality and the Levene’s test for equality of error variance. Manipulative field cage regrowth data were natural-log transformed, and natural experiment NCHO data were square root transformed. Two-way analyses of variance (ANOVA) were used to test for differences in propor-

tional change in NCHOs and total spring above-ground regrowth between treatments and between years in the manipulative field cage experiment (SPSS 11.5, SPSS Inc., Chicago, IL). Similarly, a two-way ANOVA was used to test for differences in NCHOs between years of defoliation and between sampling date in the natural experiment. When significant  $F$ -values were found, Fischer’s protected least significant difference (LSD) test was utilized to separate significant ( $P < 0.05$ ) mean differences. Mean regrowth and NCHO data were back transformed for presentation. Additionally, the percent contributions of the WSS and starch concentrations to NCHO concentration were calculated in the natural experiment.

## 3. Results

### 3.1. Manipulative field cage experiment

There were no significant differences in proportional change in NCHOs or spring regrowth between cage and no-cage controls (all  $P \geq 0.05$ ) so the no-cage controls were excluded from further analyses and only the beetle treatment and cage controls were compared. No significant treatment-by-year ( $F = 0.35$ ;  $df = 1, 31$ ;  $P = 0.56$ ) interaction effects were found for proportional changes in NCHOs, indicating the effects of cage control or beetle treatment on NCHOs did not vary with the year in which the experiment was conducted. Following defoliation, the cage control and beetle treatments did not differ significantly ( $F = 0.38$ ;  $df = 1, 31$ ;  $P = 0.54$ ) in the mean proportional change in NCHOs (Fig. 2). NCHO concentrations increased  $18.0 \pm 9.2\%$  and  $12.1 \pm 6.6\%$  in the cage control and beetle treatment trees, respectively. Analysis of absolute changes in NCHO concentrations also did not reveal significant treatment effects ( $F = 0.44$ ;  $df = 1, 31$ ;  $P = 0.51$ ). No significant treatment-by-year ( $F = 1.35$ ;

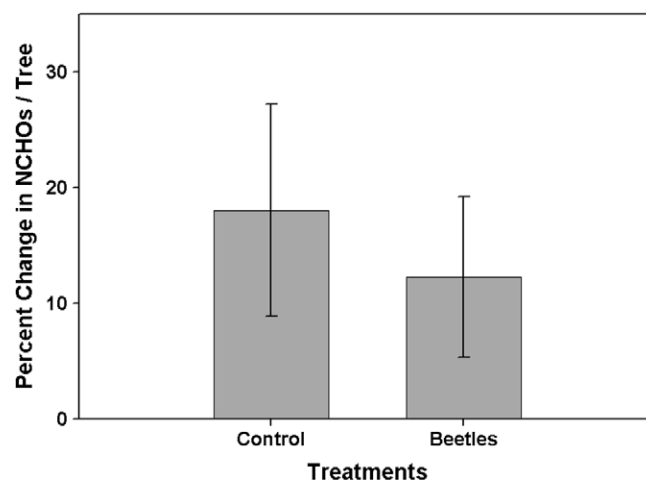


Fig. 2. Mean ( $\pm$ SE) proportional change in *Tamarix* root crown nonstructural carbohydrates (NCHOs) from the field cage experiment conducted at Lake Thomas, Texas in 2004 and 2005. Means are not significantly different ( $P \geq 0.05$ ).

$df = 1, 31$ ;  $P = 0.25$ ) interaction effects on spring above-ground regrowth were found. However, the cage control and beetle treatments did differ significantly ( $F = 6.91$ ;  $df = 1, 31$ ;  $P = 0.01$ ) in the mean spring above-ground regrowth (Fig. 3). Mean spring above-ground regrowth mass was  $135.6 \pm 17.3$  g in control trees and  $89.7 \pm 9.5$  g in trees which were exposed to beetles the previous season.

### 3.2. Non-manipulative natural experiment

Treatment (years of defoliation) by sampling date interaction ( $F = 9.20$ ;  $df = 3, 222$ ;  $P < 0.001$ ) effects on NCHO concentrations were found in the natural experiment. Because of the interaction between the main effects, comparisons of NCHOs between treatments were analyzed separately for each year. Root crown NCHO concentrations differed significantly between treatments in 2005 ( $F = 34.58$ ;  $df = 3, 102$ ;  $P < 0.001$ ) and in 2006 ( $F = 50.34$ ;  $df = 4, 118$ ;  $P < 0.001$ ). In 2005, the mean NCHO concentration was significantly less ( $P < 0.05$ ) in trees with at least one year of defoliation than in trees with no years of defoliation (Fig. 4). Mean percent NCHO concentration in trees which were not defoliated was  $9.0 \pm 0.8\%$ , compared to  $3.2 \pm 0.4\%$ ,  $2.1 \pm 0.4\%$  and  $2.3 \pm 0.4\%$  in trees defoliated for one, two and three years, respectively.

In 2006, the mean NCHO concentration was significantly less in trees which had experienced at least two years of defoliation than in trees which had experienced no more than one year of defoliation. Furthermore, NCHO concentration was significantly less in trees which had experienced one year of defoliation when compared to trees which had experienced no defoliation (Fig. 5). In 2006, mean percent NCHO concentration in trees which were not defoliated was  $13.6 \pm 0.9\%$ , compared to  $7.6 \pm 0.8\%$ ,  $2.3 \pm 0.4\%$ ,  $1.5 \pm 0.3\%$  and  $1.7 \pm 0.4\%$  in trees defoliated for one, two, three and four years, respectively. Starch contributed most

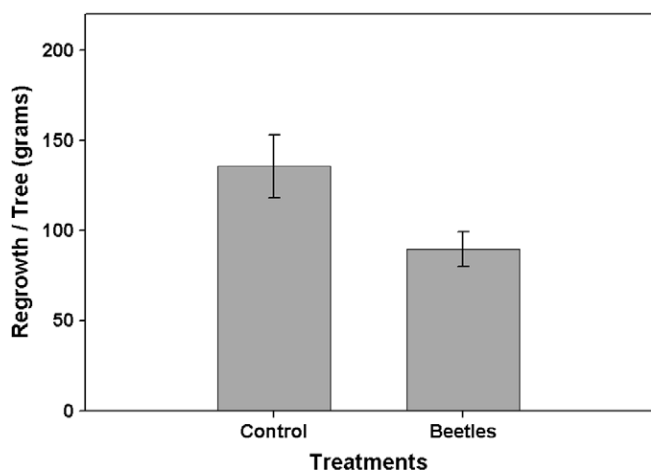


Fig. 3. Mean ( $\pm$ SE) spring above-ground regrowth of *Tamarix* trees from the field cage experiment conducted at Lake Thomas, Texas. Experiments were conducted in 2004 and 2005, and spring regrowth was measured the following spring in 2005 and 2006. Means are significantly different ( $P < 0.05$ ).

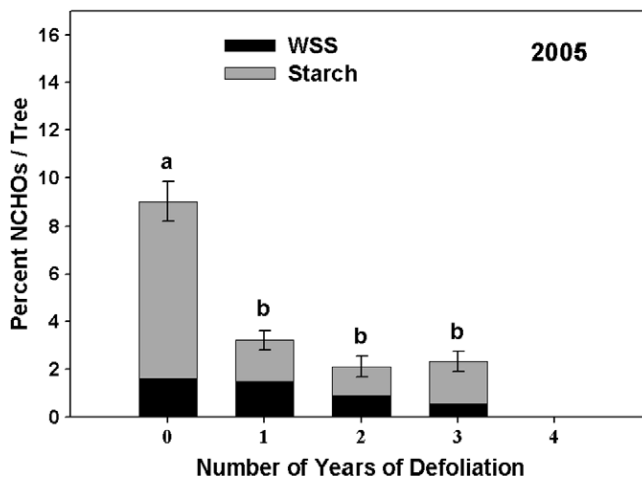


Fig. 4. Mean percent of water soluble sugars (WSS = fructose + glucose + sucrose), starch and nonstructural carbohydrates (NCHOs = WSS + starch) per *Tamarix* tree at natural experiment site near Lovelock, Nevada in 2005. Standard error bars based upon NCHOs are shown. Mean NCHO with same letter above bars are not significantly different ( $P \geq 0.05$ ).

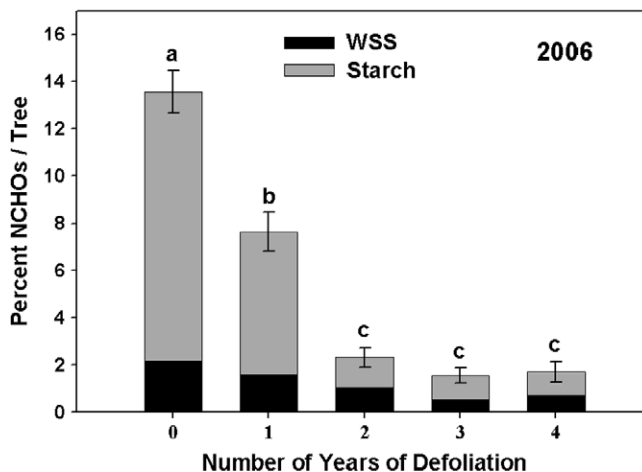


Fig. 5. Mean percent of water soluble sugars (WSS = fructose + glucose + sucrose), starch and nonstructural carbohydrates (NCHOs = WSS + starch) per *Tamarix* tree at natural experiment site near Lovelock, Nevada in 2006. The zero treatment trees were from Pyramid Lake. Standard error bars based upon NCHOs are shown. Mean NCHO with same letter above bars are not significantly different ( $P \geq 0.05$ ).

to NCHO concentrations and was the component most reduced in trees which had experienced defoliation (Figs. 4 and 5). In 2006, starch contributed 84% and 78% to NCHOs in trees defoliated for zero and one year, respectively; whereas the starch contribution was 55%, 66% and 60% in trees defoliated for two, three and four years, respectively.

## 4. Discussion

### 4.1. Manipulative field cage experiment

All experimental trees accumulated nonstructural carbohydrates in the root crowns between July and September

2004. This is in accord with the seasonal phenology of carbohydrates observed in many woody plants including *A. saccharum* (Renaud and Mauffette, 1991), *P. glandulosa* (Fick and Sosebee, 1981), *Prunus avium* L. (Clair-Maczulajty et al., 1994), *S. sebiferum* (Conway et al., 1999) and *Tamarix* (Sosebee, 2004). In general, carbohydrate reserves of storage organs decrease rapidly in early spring as the organs serve as carbohydrate sources to supply energy for budbreak, root growth and vegetative and reproductive development (Kozlowski, 1992). Reserves usually reach a maximum in the fall when acquisition via photosynthesis exceeds allocation to growth, and they then begin to decline after leaf fall and throughout the dormant season as the plants must rely on these reserves for all metabolic activity, especially respiration (Loescher et al., 1990; Kozlowski, 1992).

Although beetle treatment trees accumulated less NCHOs than did control treatment trees in the 2004 experiment (data not shown), analysis of the 2004 data alone did not detect a significant difference in the mean proportional change in NCHOs between treatments. There are two possible reasons for this result: (1) the limited sample size and high variation in NCHO data obscured the trend and (2) the fact that there was little time lag between beetle defoliation and the post-treatment sampling of the root crowns in 2004. Beetles were introduced on 16 July 2004. Very little defoliation was observed by 4 August. Trees were completely defoliated by 3 September, at which time tissue was removed from the root crown for post-treatment analysis. Richards (1993) suggested that herbivory has the strongest impact on plant growth and survival if a long delay occurs between loss of photosynthetic tissue and leaf regrowth. This suggestion may extend to the effects of herbivory on plant carbohydrate storage because the plant must draw on these stored reserves during the defoliated period. Since the beetle treatment trees were without photosynthetic tissue for less than one month before NCHOs were re-sampled, few carbohydrate reserves probably were utilized by the plants.

For the 2005 experiment, the sample size was increased to 10 per treatment and the period of time between beetle defoliation and post-treatment quantification of NCHOs was increased by sampling for NCHOs in December, four months after beetle treatment. However, beetle numbers did not increase significantly in 2005, and the trees were only lightly to moderately defoliated (all <40%) by the end of the season. The incomplete defoliation in 2005 may have contributed to the non-significant treatment effect on NCHOs. It is also possible that a single late season defoliation event does not have an immediate effect on root crown NCHOs due to compensatory effects such as carbohydrate re-allocation from the root system.

Although root crown NCHOs were not significantly affected by the single late season defoliation event, the above-ground regrowth that was measured during the following spring was significantly different between beetle treatment and control trees. Over the combined experi-

ments, beetle treatment trees manufactured approximately 35% less above-ground regrowth following one defoliation event the previous fall than did cage control trees. Reichenbacher et al. (1996) also reported significant reductions in above-ground growth parameters following manual defoliation of hybrid *Populus*, but nonstructural carbohydrates were only mildly affected when defoliation levels were between 25% and 75%. These results suggest that above-ground growth may be a more sensitive indicator of mild defoliation stress. However, barring any further defoliation stress, it is possible beetle treatment trees are able to recover from the reduction in spring regrowth. Due to the limitations in the design of this study, we are unable to state if and when the beetle defoliated trees made a recovery in regrowth.

#### 4.2. Non-manipulative natural experiment

Unlike the manipulative experiment, in the non-manipulative, natural experiment the sample trees had been defoliated for extended periods of time and under natural conditions. Extended defoliation by *D. elongata deserticola* near Lovelock, Nevada significantly reduced NCHOs in *Tamarix* root crown tissue. The significantly higher NCHO concentration in the non-defoliated trees in 2006 compared to the non-defoliated trees in 2005 raises questions regarding the use of trees from Pyramid Lake in the comparison. Although the Pyramid Lake trees were not significantly different in size, other factors (such as water and soil nutrient availability) may have influenced carbohydrate reserves compared to the sites in the Humboldt sink. Regardless, the exclusion of the Pyramid Lake trees does not negate the fact that NCHOs accounted for less than 3% of total dry weight in trees defoliated 2 or more years in 2006.

Of the individual carbohydrates quantified, starch was the predominant component to overall NCHO concentration, and it was the carbohydrate most reduced by defoliation. Starch levels were also significantly reduced in roots of *A. saccharum* trees severely defoliated by insects (Wargo et al., 1972). The authors suggested that starch levels indicate changes in carbohydrate metabolism and perhaps the magnitude of physiological disturbance. Starch is considered the most important reserve carbohydrate in woody plants because it indicates when and where a carbohydrate surplus is present above current needs (Kozlowski, 1992). In the present study, *Tamarix* trees defoliated for one to four years still had measurable quantities of starch and water soluble sugars. The presence of starch and water soluble sugars may not necessarily be an indication of continuing plant metabolism. Some carbohydrate stores may become inaccessible to woody plants with time because they are in dead cells and cannot be retrieved by the plant (Ziegler, 1964). This suggests that NCHOs can be detected in dead trees.

The depletion of *Tamarix* root crown nonstructural carbohydrates due to extensive defoliation in the natural experiment suggests a reduction in the vitality of the trees.

At Lovelock, an estimated 40% of trees in the 2-ha area which had experienced defoliation for 4 consecutive years failed to produce any foliage in 2006 and were considered dead (Dudley et al., 2006). Death of *Eucalyptus* species in Australian forests after repeated defoliation by phasmatids or psyllids, *Glacaspis* spp., was purported to be due to the exhaustion of starch reserves to a level which did not support respiration and growth (Bamber and Humphres, 1965). The European gypsy moth, *Lymantria dispar* L., increases tree mortality as the intensity, duration and frequency of defoliation increases (Davidson et al., 1999). Additional studies are required to determine the minimum level of carbohydrate storage that results in *Tamarix* death.

Carbohydrate reserves are also important to reproduction, and the depletion of these reserves in plants by herbivores has been demonstrated to reduce reproduction (Chapin et al., 1990). Flower and fruit production were significantly reduced in the perennial herb *Aralia nudicaulis* the year following herbivory by moose, *Alces alces* (Edwards, 1985). Although direct measures of the effects of defoliation on reproductive fitness were not measured in these studies, *Tamarix* trees were never reproductively active at the time of defoliation. Thus defoliation and the reduction in carbohydrate reserves by *D. elongata* herbivory may reduce recruitment of seedlings and slow the spread of *Tamarix*.

#### 4.3. Summary

Carbohydrate reserves are essential for plant survival (Loescher et al., 1990; Chapin et al., 1990). Maintenance respiration in living cells when photosynthesis is low or has stopped due to defoliation or deciduousness is dependent on adequate carbohydrate reserves, as is new spring leaf growth in all deciduous species (Loescher et al., 1990; Kozłowski, 1992). The results from the manipulative field cages experiment at Lake Thomas, indicate that a single late season defoliation by *D. elongata elongata* did not significantly affect root crown NCHOs; however, above-ground regrowth was reduced the following spring. Additional studies are needed in Texas to determine if defoliation by *D. elongata elongata* will have significant impacts on NCHOs and subsequently reduce *Tamarix* survival. *D. elongata elongata* has successfully established at a release site near Big Spring, Texas. The widespread defoliation by the beetle at this site in 2005 and 2006 affords the opportunity for additional host impact studies.

The results from the natural experiment demonstrate that extended defoliation by *D. elongata deserticola* significantly reduces nonstructural carbohydrate reserves in *Tamarix* and that repeated defoliation in subsequent years prevents recovery of these reserves. The decline in tree NCHOs appears to be associated with a reduction in foliage growth and may affect tree survival and seedling recruitment. These results indicate that *D. elongata deserticola* has a significant host impact and suggests there is the

potential for successful control of *Tamarix* at the Nevada site.

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