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Biological control reduces growth, and alters water relations of the saltcedar tree (*Tamarix spp.*) in western Nevada, USA

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ABSTRACT

We monitored the impacts of a biological control agent, the saltcedar leaf beetle (*Diorhabda carinulata*), on the saltcedar tree (*Tamarix* spp.) at two sites (Humboldt and Walker rivers) in Nevada, USA. At the Humboldt site trees that had experienced three to four defoliation events had more negative water potentials and lower foliar $\Delta^{13}C$ than trees farther from the release site that had experienced only one defoliation event. We established paired trees (exposed to *D. carinulata* and sprayed with insecticide) at both sites and monitored impacts. Beetles reduced stem growth during the first year of defoliation at both sites but not in the second year at the Humboldt site. Defoliation did not affect midday water potentials, or leaf gas exchange during the first two years of defoliation of paired trees at either site. Furthermore there was no difference in foliar $\Delta^{13}C$ in either year at the Humboldt site but defoliation during the first year lead to higher foliar $\Delta^{13}C$ at the Walker site. These results suggest that initial defoliation by *D. carinulata* reduces growth but not water relations of saltcedar. However, repeated defoliation, potentially acting through reduced root growth, leads to an overall reduction in the water status of this invader.

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1. Introduction

Saltcedar (*Tamarix* spp.) is a deciduous shrub or small tree native to Eurasia and Africa that has invaded riparian and wetland habitats throughout the arid and semiarid western USA (Robinson, 1965; Dudley et al., 2000; Glenn and Nagler, 2005). Introduced into the USA in the early 19th century, it now occupies >650,000 ha (cf. Zavaleta, 2000) and appears not to have reached its full potential distribution in North America (Morisette et al., 2006). The success of this facultative phraetophyte is attributed to its ability to maintain favorable water relations in these water limited systems (Glenn and Nagler, 2005 and references therein).

Biological control is one method for controlling the spread and impacts of unwanted plants (Hoffmann and Moran, 1998; Moran et al., 2005). Yet the actual ecological outcomes of biological control on communities and ecosystems are often not well studied and success is often defined as a reduction in the abundance of the target organism (Denslow and D'Antonio, 2005; Thomas and Reid, 2007). In instances where the target organism is a perennial species it may be difficult to document immediate reductions in population abundance. Studies examining the impacts of biological control on growth or a key aspect of a plant's physiology may provide insights into its ability to reduce overall plant performance.

The saltcedar leaf beetle *Diorhabda carinulata* (Brullé) *sensu lato* (Coleoptera: Chrysomelidae) (Tracey and Robbins 2009) was approved for release in the USA in 1996 to assist in efforts to control saltcedar (Dudley et al., 2000; DeLoach et al., 2003; Lewis et al., 2003). This foliage feeder has been introduced into much of the western USA with varying establishment success (DeLoach et al., 2003). To date there have been relatively few studies on the impacts of *D. carinulata* outside of cages and no published studies on the potential impacts this agent may have on saltcedar growth and physiology. In this study we examined *D. carinulata* and saltcedar interactions in two western Nevada sites where *D. carinulata* was introduced, has successfully established and has defoliated trees over several years. Because water is a limiting resource to

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plant growth in this and other regions where saltcedar occurs we measured the impacts of *D. carinulata* on saltcedar growth and water relations. Of additional importance to understanding the impacts of *D. carinulata* on saltcedar is the recent characterization of saltcedar as a potentially beneficial species (Stromberg et al. 2009). Our study occurred both across a chronosequence of defoliation resulting from *D. carinulata* spread across the landscape and in trees treated with insecticide to minimize defoliation by this herbivore.

Herbivory can increase root to shoot ratios resulting in increased leaf level transpiration and improved whole plant water status (Heichel and Turner, 1983; McNaughton, 1983; Welker and Menke, 1990; Reich et al., 1993; Houle and Simard, 1996; Alstad et al., 1999). Extended herbivory however, may also result in reduced root growth which can reduce plant drought tolerance and overall water status (Snyder and Williams, 2003). In this study we measured the impacts of *D. carinulata* on stem growth, midday water potentials, and integrated and instantaneous leaf gas exchange on trees that had experienced varying levels of defoliation. Results of this study provide insights into the early effects of saltcedar biocontrol on the growth and water relations of saltcedar in stands throughout western North America (DeLoach et al., 2003; Morisette et al., 2006).

2. Materials and methods

2.1. Study sites

Diorhabda carinulata were released at single locations in saltcedar stands on the lower reaches of the Humboldt and Walker Rivers in central Nevada, USA in the late spring of 2001. The Humboldt River (hereafter, Humboldt site) and Walker River (hereafter, Walker site) sites are characterized by fine, saline silt soils from fluvial deposits within active and remnant, moderately incised river channels. The annual precipitation at the Humboldt site is 14 cm and at the Walker site it is 12 cm.

Diorhabda carinulata overwinter as adults under the litter of saltcedar and emerge in late April to mid May (Lewis et al., 2003). Both larvae and adults feed on leaf foliage and petioles which results in desiccation and eventual loss of leaves from trees. Emerging adults typically move in large congregations towards available foliage (Lewis et al., 2003). Saltcedar trees at these sites typically produce the first foliage in April and experience senescence in November, however earlier senescence can occur if trees experience cold temperatures. Trees are often affected by a single major defoliation event in the growing season followed by lesser amounts of defoliation later in the season and will produce new foliage after defoliation within a season.

Dispersal of *D. carinulata* was determined from ground surveys conducted at 1–4 week intervals and by survey flights conducted 1–2 times per growing season from 2002 until 2005. Surveys showed an expanding pattern of defoliation within and across seasons. At the Humboldt site we worked in three areas of defoliation: (1) The Humboldt 2002 Defoliated Area (2002DA) included the release site and the 2 ha area subsequently defoliated in 2002. Trees in the 2002DA had experienced four consecutive defoliation events by July 2004. (2) The 2003DA consisted of trees outside of the 2002DA that were first defoliated in 2003. The total area of defoliation in 2003 was approximately 200 ha and extended up to 2 km away from the release site. Trees in the 2003DA had experienced three consecutive defoliation events by July 2004. (3) The 2004DA consisted of trees outside the other areas that were first defoliated in 2004. The total extent of defoliation in 2004 was approximately 1800 ha (Geraci, 2006) and included trees up to 25 km from the release site. Trees in the 2004DA had experienced a single defoliation event by July 2004. In 2005 *D. carinulata* fed to varying extents on trees in all previously defoliated areas. We randomly selected trees within single locations (100 m radius) in each of the three areas (2002DA, 2003DA and 2004DA) at the Humboldt site. Selection of locations was based on accessibility and similarity with surrounding stand characteristics. The location in the 2002DA was adjacent to the release site. The location in the 2003DA occurred approximately 200 m beyond the 2002DA and the 2004DA location occurred approximately 0.5 km beyond the 2003DA.

At the 2004DA location we established 12 pairs of trees in May, 2004. Trees in each pair were within 4–10 m of each other and were similar in height, diameter and canopy shape and cover. One tree in each pair received an insecticide treatment (see below) and the other was exposed to *D. carinulata*. Heights of paired trees ranged from 2.5 to 3.0 m. Tree ages were likely similar as observations of the timing of stand establishment suggest that the vast majority of trees in the area established after a major flooding event in 1984 (A. Brinkerhof pers. comm.).

At the Walker site *D. carinulata* were released in April, 2001 and by September had established on 10–20 trees near the release site. In September, 2003 *D. carinulata* spread and defoliated most of the trees within approximately 2.5 km from the release site. In 2005 *D. carinulata* spread and defoliated the entire southern extent of the Walker riparian area up to 9 km away from release site. We established a location near the edge of the stand in May 2005 approximately 5 km south of the release site and 1.8 km from the Walker River. At this location we set up 10 pairs of trees where one in each pair was exposed to and one protected from *D. carinulata* using insecticide (as at Humboldt).

Paired trees in each site were treated in the following way: One member of each pair was randomly selected to be sprayed with the commercial insecticide 4F carbaryl (trade name SEVIN, Bayer Crop Science, Research Triangle Park, North Carolina) to reduce defoliation. The concentration of 4F carbaryl was 22.5% by weight and was applied at a dosage of 44 ml insecticide to 3785 ml water. Trees were sprayed as often as necessary (3–5 times a growing season) to minimize *D. carinulata* defoliation. A small amount of defoliation occurred on these trees due to some herbivore colonization between spray events.

In each of the three locations at the Humboldt site a single shallow well was installed. Wells consisted of a 2.5 cm diameter steel pipe with 50, 1.5 cm slots cut into it within the bottom 1 m. Depth to the water table was determined biweekly with electric tape from mid June to October of 2004 and 2005. A well was not installed at the Walker site.

2.2. Stem growth

The impacts of *D. carinulata* on stem growth were determined by repeated measurements of two similarly sized stems (1.5–2.0 cm) of similar height and orientation on each of the paired trees (*D. carinulata*-exposed and insecticide-treated) at the Humboldt and Walker sites. Flagging and ink were used to identify locations on stems where measurements were made. Each stem's diameter was determined from two measurements to the nearest 0.01 mm made in orthogonal directions using calipers. Changes in stem diameter relative growth rate (stemRGR) over time were determined with the equation:

$$stemRGR = \frac{d_2 - d_1}{t_2 - t_1} \quad (1)$$

where d_2 is the ln of stem diameter at time t_2 and d_1 is the ln of stem diameter at time t_1 and where t_2 and t_1 represent the final and initial measurement periods respectively (Hoffmann and Poorter, 2002). The stemRGR for each tree was determined from the

average of the two stems. At the Humboldt site, stemRGR was determined for the periods of July 6, 2004 (t_1) to June 9, 2005 (t_2) and for June 9, 2005 (t_1) to October 28, 2005 (t_2). At the Walker site, stemRGR was determined for the period of June 10, 2005 (t_1) to October 26, 2005 (t_2). StemRGR over the two time periods at the Humboldt site were compared separately because the first time period included time during dormancy while stemRGR in the second time period (summer 2005) occurred entirely during the growing season. StemRGR at the Walker site was compared between the two herbivore exposure treatments separately from the Humboldt site because of differences in the timing of *D. carinulata* defoliation. Data for the Humboldt site in 2004 were analyzed with ANOVA (proc GLM, SAS). Data for the Humboldt and Walker sites in 2005 failed normality in spite of transformations and were therefore analyzed with separate Mann Whitney *U* tests (SPSS, 2002).

2.3. Stem water potential

Midday stem water potentials (Ψ_{md}) were measured with a Scholander-type pressure chamber (PMS Instruments, Corvallis, Oregon, USA) to determine if *D. carinulata* defoliation impacted whole tree water status. Measurements were taken between 12:30 and 14:30 on terminal twigs of trees. Measurements at the Humboldt site occurred in the three locations (2002DA, 2003DA and 2004DA) on different trees on June 15, 2004 ($n = 8$ trees per area); September 9, 2004 ($n = 4$ trees per area) and on July 18, 2005 ($n = 8$ trees per area). Measurements on the paired trees at the Humboldt site occurred on September 7, 2004 ($n = 5$), July 18, 2005 ($n = 8$) and September 13, 2005 ($n = 6$). At the Walker site measurements occurred on all ten pairs of trees on July 26, and August 22, 2005. A two-way ANOVA (Proc GLM, SAS) (with area and date as factors) was used to test for differences in Ψ_{md} between areas (2002DA, 2003DA and 2004DA) within dates at the Humboldt site. We used repeated measures ANOVA (Proc Mixed, SAS) to compare the *D. carinulata*-exposed and insecticide-treated paired trees in the Humboldt and Walker sites separately.

Foliar Δ^{13}C and N- The Δ^{13}C of foliage was used to provide an integrated measure of internal CO_2 concentration from which inferences about stomatal openness and leaf level water use could be made (Dawson et al., 2002). Trees were compared in each of the three areas at the Humboldt site and comparisons were also made between the paired trees at both Humboldt and Walker sites. Trees from the 2002DA, 2003DA and 2004DA's were sampled in August 26, 2004 ($n = 13$, 16 and 21 trees respectively) and again in July 18, 2005 ($n = 5$, 6 and 6 trees, respectively). Different trees were used for each of these collections. The paired trees at the Humboldt site were sampled on September 16, 2004 ($n = 5$) and July 18, 2005 ($n = 10$). The paired trees at the Walker site were sampled on August 22, 2005 ($n = 10$). In all cases, the youngest fully expanded foliage was sampled on the south side of trees. Foliage was dried at 55 °C for 3–4 days and then ground to a fine powder in a ball mill. Samples containing 1–2 mg of powdered material were loaded into tin capsules and sent to the University of Georgia Stable Isotopes Laboratory (Athens, Georgia, USA). Samples were analyzed for total N using the Dumas micro-combustion technique (Carlo-Erba Model NA 1500, Strumentazione, Milan, Italy) and then analyzed for stable carbon isotope composition (δ^{13}C) on a mass spectrometer. The values of δ^{13}C were calculated from

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \quad (2)$$

where R_{sample} is the absolute isotopic ratio of ^{13}C to ^{12}C and $R_{standard}$ is the respective ratio of a standard (Craig, 1957). A value of atmospheric δ^{13}C of −8‰ was used to calculate discrimination of ^{13}C (Δ^{13}C) using the formula by Farquhar et al. (1989):

$$\Delta^{13}C = \frac{1000\left(\delta^{13}C_{air} - \delta^{13}C_{leaf}\right)}{1000 + \delta^{13}C_{leaf}} \quad (3)$$

Leaf N levels from these same samples were used to indicate potential differences in leaf mineral nutrition associated with defoliation and to assess potential differences in photosynthetic capacity for interpretations of Δ^{13}C (Evans et al., 1986). Values of Δ^{13}C and N at the 2002DA, 2003DA, 2004DA in 2004 and 2005 at the Humboldt site were compared using two-way ANOVA (PROC GLM, SAS) with area and date as factors. Paired trees at the Humboldt site in 2004 and 2005 were compared using repeated measures ANOVA (Proc Mixed, SAS). Paired trees at the Walker site (2005) were compared using a one way ANOVA (Proc GLM, SAS). Because of differences in the timing of *D. carinulata* arrival, Humboldt and Walker trees were compared separately.

2.4. Leaf gas exchange

We measured leaf photosynthetic rate (A), stomatal conductance (gs), and leaf transpiration (E) of the paired trees at the Humboldt and Walker sites. Measurements were made on the youngest fully mature leaf of $n = 4$–6 pairs of trees between 8:00 and 11:00 on clear days. Measurements at the Humboldt site occurred on September 15, 2004, July 25, 2005 and September 13, 2005. Measurements at the Walker site occurred on July 26 and August 18, 2005. For measurements in 2004 (Humboldt site) an LI-6200 (LiCor, Lincoln, NE, USA) gas exchange system was used with measurements occurring on clear sunny days at ambient light levels. All measurements in 2005 were made with an LI-6400 system with a light source set at 1800 μmol/m^2/sec of PAR and CO_2 levels set at 380 ppm. Leaf area in the chamber was determined with an LI-3100 leaf area meter (LiCor, Lincoln, NE, USA). Leaf gas exchange data from each site were analyzed separately with repeated measures analysis (Proc Mixed SAS). Leaf photosynthetic rates were used to provide insights into the relative roles of stomatal closure and photosynthetic demand on Δ^{13}C values of the paired trees (Evans et al., 1986).

In all ANOVA analyses type III sums of squares and least squares means were used to determine levels of significance because of missing data. We tested the residuals from all analyses for normality and homogeneity of variance. Least squares means were used to test for significant differences. Levels of significance were determined at $P < 0.05$ with adjustments made to alpha levels for multiple post hoc comparisons. SAS ver 9.1 was used for all SAS analyses (SAS, 2003).

3. Results

3.1. Stem growth

Defoliation strongly and significantly reduced stemRGR: at the Humboldt site in the first time interval (July 6, 2004 to June 9, 2005) RGR was -7.7×10^{-3} ($\pm 23.8 \times 10^{-3}$) and 99.2 × 10-3 ($\pm 20.0 \times 10^{-3}$) mm/mm/yr for *D. carinulata*-exposed versus insecticide-treated trees respectively ($F_{1,14} = 11.79$; $P < 0.001$). The values of stemRGR in the second interval (June 9 to October 28, 2005) were 1.8×10^{-4} ($\pm 1.4 \times 10^{-4}$) versus 29.5×10^{-4} ($\pm 16.5 \times 10^{-4}$) mm/mm/day for stems on *D. carinulata*-exposed and insecticide-treated trees respectively. While this follows the same pattern of insect-induced growth reduction, the means were not significantly different due to wide variation in growth in the

insecticide-treated trees ($U = 5.00$, $P = 0.151$). The stemRGR of the paired trees at the Walker site ($n = 10$) from June 10 to October 26, 2005 (138 days) was -6.3×10^{-4} ($\pm 4.9 \times 10^{-4}$) and 5.6×10^{-4} ($\pm 1.8 \times 10^{-4}$) mm/mm/day for *D. carinulata*-exposed and insecticide-treated trees respectively ($U = 9.00$, $P = 0.001$). Negative stemRGR on *D. carinulata*-exposed trees likely reflects the combination of little or no growth, slight variation associated with placement of calipers on stems and perhaps shrinkage of the stems due to desiccation after defoliation.

3.2. Stem water potential

At the Humboldt site trees close to the release site (2002DA) tended to have more negative midday water potentials than trees in the other areas (Fig. 1). There was a significant effect of area ($F_{2,55} = 28.94$, $P < 0.001$) but not date ($F_{2,55} = 0.98$, $P = 0.383$) and area effects were consistent across date (interaction $F_{4,55} = 0.82$, $P = 0.517$). The effect of beetles on midday water potential of paired trees varied across dates (date x defoliation interaction $F_{2,18} = 4.17$, $P = 0.0325$, Fig. 2A). Defoliation initially (Sept. 2004) appeared to increase water availability to the plants whereas later there were no clear effects. At the Walker site a marginally significant ($F_{1,18} = 3.46$, $P = 0.0794$) effect of defoliation was seen at both time points with defoliation leading to less negative water potentials and water potential increasingly slightly over time (date $F_{1,18} = 15.95$, $P < 0.001$) (Fig. 2B).

Foliar $\Delta^{13}C$ and N- The $\Delta^{13}C$ values of leaves at the Humboldt site showed a significant ($F_{2,62} = 48.43$, $P < 0.001$) effect of area (2002DA, 2003DA and 2004DA) that was consistent over time (date $F_{1,62} = 0.00$, $P = 0.9601$; interaction $F_{2,62} = 0.65$, $P = 0.5251$) (Fig. 3A). Post hoc tests (Tukeys adjusted HSD) revealed differences between the three areas (Fig. 3A) with much less discrimination occurring in leaves of trees near the release site (2002DA) compared to the other areas. For the paired trees at the Humboldt site there was a small effect of defoliation ($F_{1,8} = 5.23$; $P = 0.0515$) that was consistent over time (interaction $F_{1,8} = 0.83$, $P = 0.3886$) (Fig. 3C). At the Walker site, among the 10 paired trees those that were sprayed with insecticide had significantly ($F_{1,18} = 18.41$; $P < 0.001$) lower $\Delta^{13}C$ than trees with *D. carinulata* (Fig. 3C).

There was no correlation between $\Delta^{13}C$ and foliar N for any of the sets of trees other than a weak negative relationship for paired trees at the Humboldt site in 2004 ($P = 0.050$, $r^2 = 0.14$, $\Delta^{13}C = 218 - 0.772$ %N). This suggests that differences in $\Delta^{13}C$ were not attributable to differences in carbon assimilation. Leaf N of leaves at the three areas (2002DA, 2003DA and 2004DA) at Humboldt were not significantly different ($F_{2,56,} = 0.22$, $P = 0.8019$) (Fig. 3B). Leaf *N* values were lower in 2005 than 2004 ($F_{1,56} = 6.18$, $P = 0.0159$) and this trend was consistent across all three areas (interaction $F_{2,56} = 2.21$, $P = 0.1190$). For the paired trees at the Humboldt site there was not a consistent effect of defoliation ($F_{1,17} = 0.13$, $P = 0.7184$) or date ($F_{1,17} = 0.09$, $P = 0.7724$) (Fig. 3D). Indeed, the effect of defoliation on leaf N was opposite in the two measured dates(interaction, $F_{1,17} = 4.29$, $P = 0.0540$) (Fig. 3D). Defoliation did not alter leaf N of the paired trees at the Walker site ($F_{1,17} = 0.52$, $P = 0.4809$). Tests involving spraying the same foliage type indicated that insecticidal spray did not alter the $\Delta^{13}C$ or N content (Pattison unpublished data).

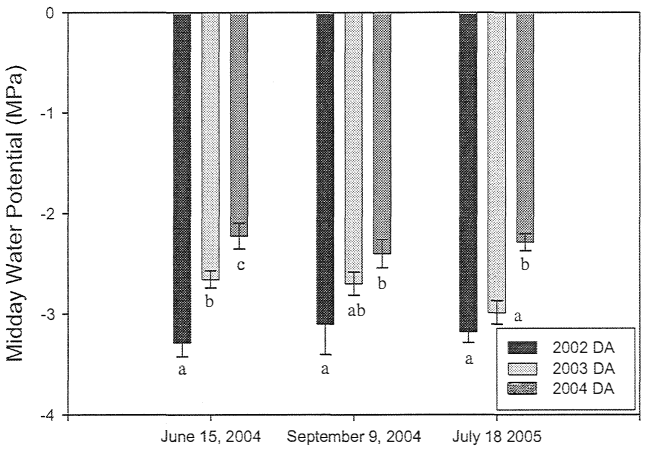

Fig. 1. Midday water potentials of trees from each of three areas representing a chronosequence of defoliation at the Humboldt site. Bars are the means of 4–8 trees and ±1 s.e. Bars with different letters within a date are significantly ($P < 0.05$) different.

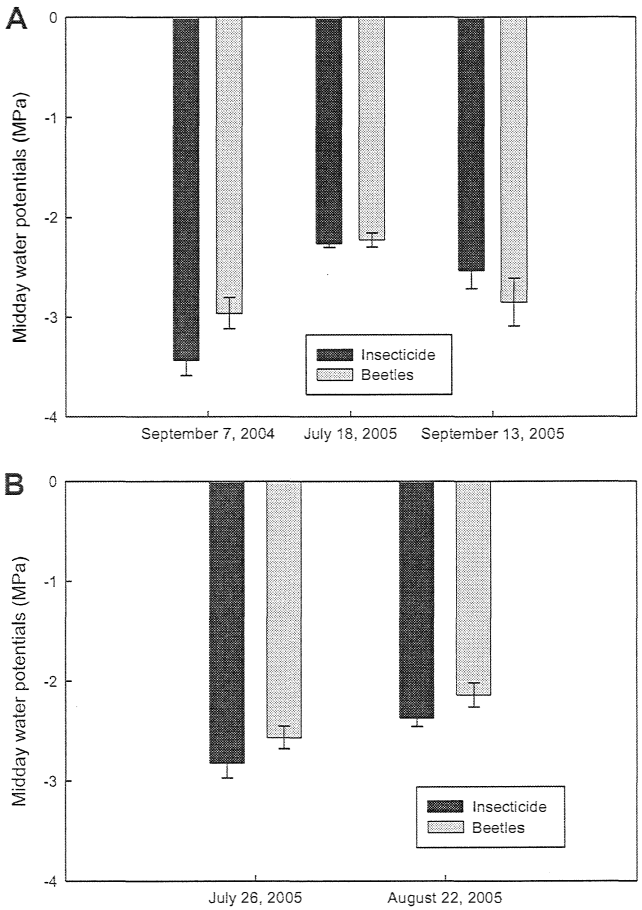

Fig. 2. Midday water potentials of paired trees exposed to biological control and sprayed with insecticide at several dates from A. the Humboldt site and B. the Walker River site. Bars are the means of 5–10 trees and ±1 s.e.

3.3. Leaf gas exchange

Defoliation did not significantly impact instantaneous measures of leaf gas exchange for trees at either site Fig. 4).Values for transpiration and conductance were significantly lower in mid summer at both sites (Fig. 4). None of these leaf gas exchange parameters were correlated with leaf or air temperature at either site.

3.4. Depth to ground water

The depth to ground water during 2004 varied from 2.5 to 2.6 m, for the 2002DA, 2.2–2.3 m for the 2003DA, 4.2–4.3 m for the

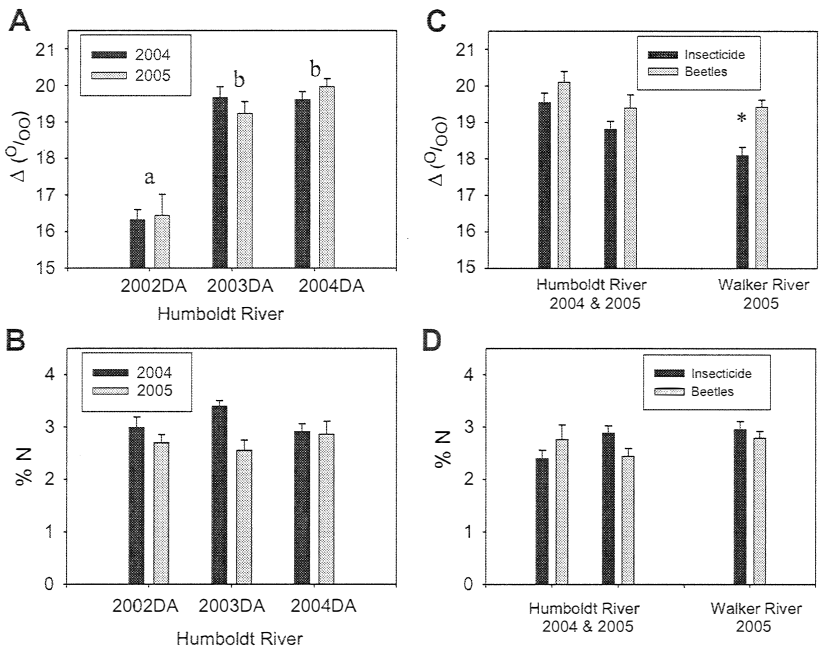

Fig. 3. Leaf carbon isotope discrimination values (A) and % N (B) from three areas representing a chronosequence of defoliation at the Humboldt River site in August 26, 2004 and July 18, 2005. Leaf carbon isotope discrimination values (C) and % N (D) from paired trees at the Humboldt site in September 16, 2004 and July 18, 2005 and the Walker River site on August 22, 2005. Letters represent significant differences ($P < 0.05$) between the three areas when pooled across dates. The * indicates a significant ($P < 0.05$) difference between trees exposed to biological control and those sprayed with an insecticide.

2004DA and from 4.5 to 4.8 m for the paired trees. In 2005 depths were completely overlapping in all areas. Depths ranged from 1.7 to 2.5 m for the 2002DA, 1.4–1.85 m for the 2003DA, 1.3–1.8 m for the 2004DA and 1.5–2.5 m for the paired trees.

4. Discussion

This study of the impacts of the saltcedar leaf beetle, *Diorhabda carinulata* during the initial (one to five years) stages of

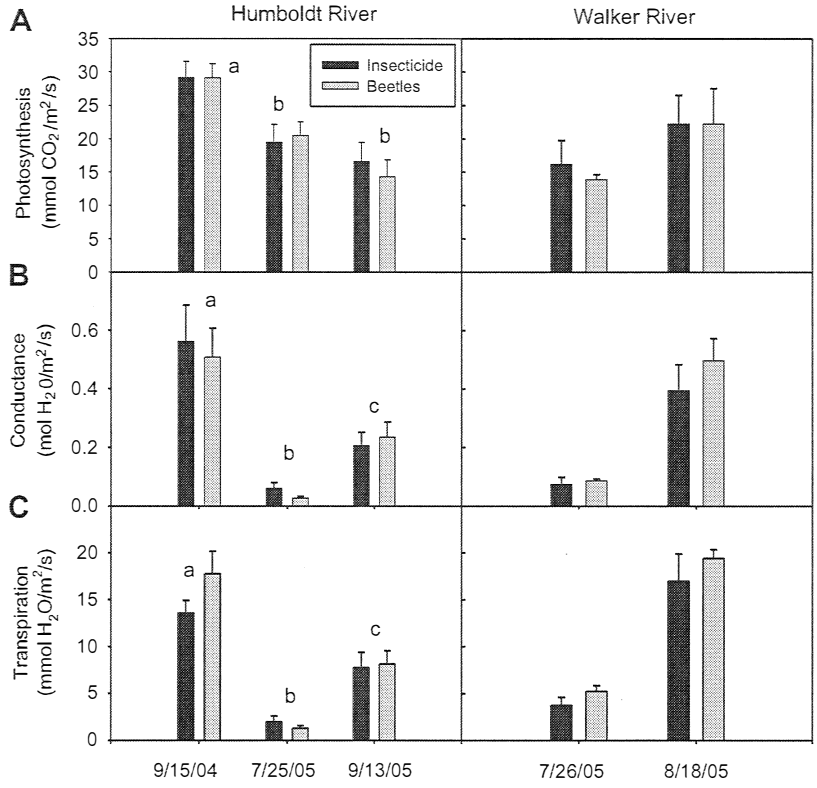

Fig. 4. Values of A. photosynthetic rates, B. conductance, and C. leaf transpiration and the Humboldt and Walker River Sites. Bars are the means (+SE) of $n = 4–6$ pairs of trees in which one member of each pair was sprayed with an insecticide. Different letters indicate significant ($P < 0.05$) differences between measurement dates within a site.

establishment and spread demonstrated the following. During the first one to two defoliation events trees experienced reduced stem growth that was dependent on beetle numbers, but there was no impact on water relations or gas exchange. In fact defoliation improved the water status of some trees. However after repeated (e.g., four) defoliation events trees experienced reduced water status potentially as a result of reduced allocations to root growth.

Beetles decreased stem growth of paired trees during the first year of defoliation at both sites but not in the second year at the Humboldt site. This is likely to be due to a reduction in *D. carinulata* abundance in the second year in this area. In separate study (Pattison et al., in press) we monitored the canopy cover of trees and abundance of *D. carinulata* at the Humboldt site from 2004 to 2005. The average number of larvae per *D. carinulata*-exposed tree of the paired trees at the Humboldt site was 276.6 (±91.6) in the first year of defoliation (2004) but only 12.9 (±2.4) in 2005. Likewise the loss of canopy in that year was less (66% in 2004 versus 47% in 2005).

Stem water potential and foliar $\Delta^{13}C$ data suggest a pattern consistent with other studies (Heichel and Turner, 1983; Welker and Menke, 1990; Houle and Simard, 1996; Alstad et al., 1999) in that initially defoliation may improve whole plant water status (Fig. 2B) and increase leaf level water use (Fig. 3B) possibly by temporally increasing root to shoot ratios (McNaughton, 1983; Reich et al., 1993; Alstad et al., 1999). However continual reduction of shoot biomass as had occurred in the 2002DA may lead to a concurrent reduction in root biomass and a decline in overall water status (Snyder and Williams, 2003). Manual defoliation decreased the ability of another facultative phreatophyte, Velvet mesquite (*Prosopis velutina*), which can co-occur with saltcedar, to access deeper sources of water (Snyder and Williams, 2003). The long term consequences of repeated defoliation of trees may therefore be reduced capacity to access deeper sources of water (Snyder and Williams, 2003) and lower nutrient uptake (Peinetti et al., 2001). In our study trees closest to the release site had more negative water potentials (Fig. 1A) and lower $\Delta^{13}C$ values (Fig. 3A) despite the same depth to water table as the 2003DA and 2004DA in 2005. These results are consistent with this mechanism. The lower $\Delta^{13}C$ values are potentially the result of increased stomatal control of water loss in response to water stress as seen in other studies with saltcedar (Cleverly et al., 1997; Devitt et al., 1997; Horton et al., 2001a; Mounsif et al., 2002). The lack of a difference in foliar N among trees across these areas suggests that trends in $\Delta^{13}C$ were not driven by differences in photosynthetic capacity. The canopy cover of trees in the 2002DA was reduced by 95% relative to non-defoliated trees after the third year of defoliation (Pattison et al., in press). There was no evidence that other site specific factors contributed to differences in water potentials and $\Delta^{13}C$ values. The terrain at Humboldt site is flat and the stand of saltcedar is relatively homogenous and of similar age (A. Brinkerhoff pers. comm.). Depth to ground water was shallowest at the 2002DA and 2003DA; locations where trees showed the most signs of water stress. None of the depths to ground water measured at the Humboldt site are likely to limit saltcedar water relations. Unlike native riparian trees which showed declines in photosynthesis and stomatal conductance with deeper ground water, saltcedar trees showed no response at two rivers in Arizona where water tables were as deep as 4–7 m (Horton et al., 2001b). In a separate study these same authors found declines in photosynthesis, stomatal conductance and foliar $\Delta^{13}C$ in saltcedar only when depths to ground water reached 6–7 m (Horton et al., 2001a).

Horton et al. (2001c) found that net photosynthesis and stomatal conductance of saltcedar were reduced when midday water potentials were lower than −2.8 MPa and −2.5 MPa respectively. Based on these results, *D. carinulata* defoliation likely reduced both of these gas exchange parameters in the 2002DA and 2003DA but not the 2004DA (Fig. 1). The $\Delta^{13}C$ data suggest that impacts on leaf gas exchange occurred on trees in the 2002DA but not the other two areas (Fig. 3A).

The lack of difference in gas exchange parameters during either year but particularly during the first year of defoliation when we would expect it most is potentially attributable to the following: midday stomatal closure resulting in a lack of sensitivity of instantaneous measures of gas exchange to variations in water status, or variability in gas exchange measures with environmental conditions not incorporated into these measurements. The results of foliar $\Delta^{13}C$ from the paired treatment suggest that these values were trending towards improved water status during the early defoliations at both the Humboldt and Walker sites even though they were only significantly different at the Walker site. This indicates that the integrated measures of water status measured with $\Delta^{13}C$ were more effective at incorporating changes in water status than instantaneous measures.

The success of saltcedar is often attributable to its greater tolerance of water stress than native species (Glenn and Nagler, 2005 and references therein). Our results suggest that repeated defoliation reduces the growth and water relations of saltcedar. These occur potentially through decreases in allocation to root biomass. Additional support for this hypothesis comes from Hudgeons et al. (2007) who found that nonstructural carbohydrate reserves in the root crowns of trees in the 2002DA and 2003DA, measured in 2005 were significantly lower (79–89%) than those in the 2004DA or in non-defoliated trees. Successful long term establishment of *D. carinulata* is therefore likely to be effective in reducing the growth of saltcedar. We believe however, that it is unlikely that *D. carinulata* will result in large scale eradication of saltcedar because even after years of defoliation most of the trees in this study are still alive. Further research is needed to understand how *D. carinulata* will impact water relations and growth over the long term and in different communities where saltcedar has invaded. For example of interest would be understanding if defoliation reduces root growth and changes source water usage across a range of communities.

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Appendix. Supplementary material

The supplementary data associated with this article can be found in the online version at doi:10.1016/j.jaridenv.2010.11.006.

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