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Managing Water in the West

Technical Memorandum No. 86-68220-10-04

Effects of the Biological Control Agent *Diorhabda elongata deserticola* on Resprouted Saltcedar

Pueblo, Colorado
2008 Final Report

U.S. Department of the Interior
Bureau of Reclamation
Technical Service Center
Denver, Colorado

March 2011

Mission Statements

The mission of the Department of the Interior is to protect and provide access to our Nation's natural and cultural heritage and honor our trust responsibilities to Indian Tribes and our commitments to island communities.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

Acknowledgements

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Pueblo, Colorado
2008 Final Report

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Introduction

In 2001, the saltcedar biological control agent *Diorhabda elongata deserticola*, a beetle originating in Fukang, China, was released at a site near Pueblo, Colorado (Eberts et al. 2005). In June of 2000, Bureau of Reclamation's Technical Service Center (TSC) began monitoring to determine effects of the biocontrol on the target species and indirect effects to secondary vegetation (Siegle and Hosler 2010). The project area comprised approximately 10 hectares at the base of Pueblo Dam on the Arkansas River (Figure 1). Initial monitoring included 100 mature saltcedar trees, which was reduced to 41 trees in 2003. This site was monitored through 2007 and is shown in Figure 2 as "Original tree stand". The original saltcedar stand was removed in spring of 2008. Results from this study found that although the biocontrol agent did heavily impact foliage, data collected could not confirm an impact to the overall growth and reproduction of saltcedar.

In 2004, the State of Colorado implemented a saltcedar management program that involved mechanically removing saltcedar in areas surrounding the original study area. These control efforts provided an opportunity for TSC to examine the effects the biocontrol agent might have on resprouted saltcedar and if there would be greater impacts to younger resprouted saltcedar than to mature saltcedar assessed in the original study (Siegle and Hosler 2010). The idea for the resprout study materialized when a number of beetles were observed foraging on the

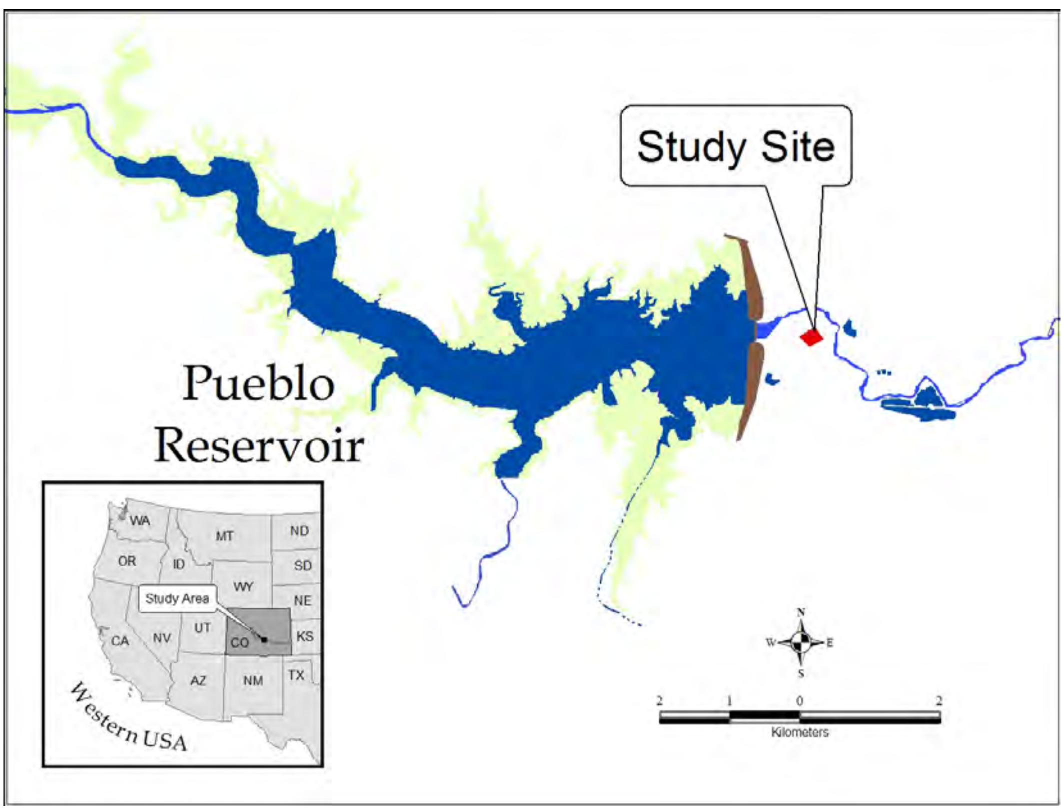


Figure 1.—Location map of saltcedar biocontrol vegetation monitoring site; Pueblo, CO.

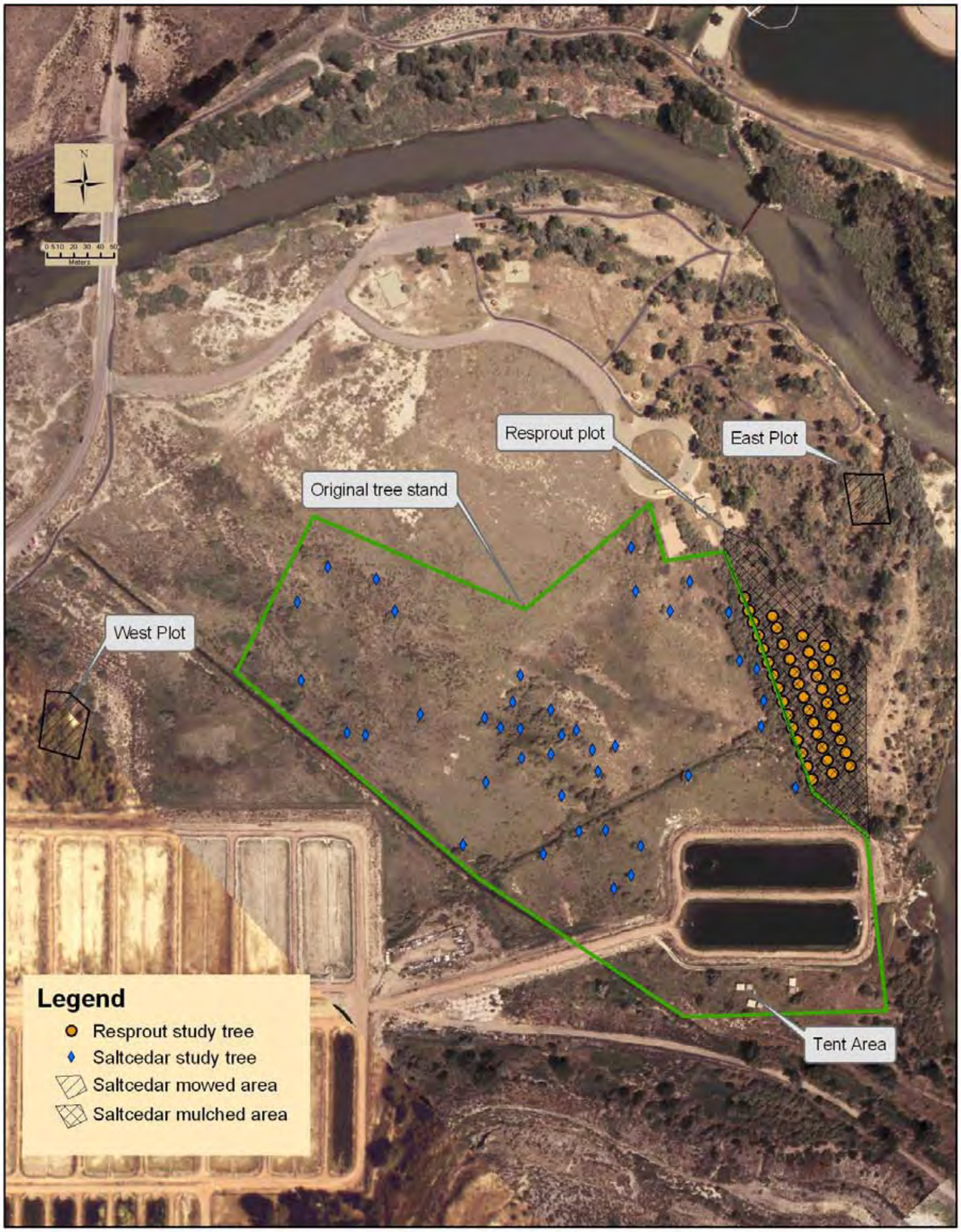


Figure 2.—Saltcedar biocontrol agent vegetation monitoring site; Pueblo Colorado.

resprouted saltcedar foliage in the recently treated area, but very few beetles were detected in the original site. Three study plots were created: Resprout Plot and East and West Plots (Figure 2). These auxiliary studies are the subject of this report.

Methodology was slightly different between the Resprout Plot and the East and West Plots. Baseline data were collected prior to treatment within the Resprout Plot with the objectives of: 1) examining the potential for saltcedar to resprout following mechanical treatment, 2) determining if the health of saltcedar prior to treatment influenced resprouting potential or resistance to the biocontrol agent, 3) observing and recording beetle feeding preferences, and 4) assessing effects of the beetle on saltcedar that resprouted within this larger plot. The objectives of the East and West Plot study were to 1) examine effects of the biocontrol agent on saltcedar post-treatment and 2) observe and record beetle feeding preferences. Monitoring at the Resprout Plot was conducted from October of 2004 through September of 2008. Monitoring within East and West plots was conducted from June 2005 through September 2008. The East and West Plots were treated prior to the Resprout Plot, and post-treatment data collection began one year earlier in these plots than in the Resprout Plot.

Methods

Resprout Plot

TSC established a plot of approximately 1 hectare at this site (see “Resprout plot” in Figure 2), which was mulched in July of 2006 (Figure 3). Mulching equipment removes saltcedar at the root crown, disrupting the meristematic tissue, while simultaneously grinding material into fine segments. Prior to treatment, the stand was composed of primarily mature, decadent saltcedar. Baseline data was collected in the fall of 2004 and 2005, which entailed identifying 44 saltcedars and documenting location and general information on size and health. Post-treatment, locations of all saltcedar within the hectare plot were recorded with the Trimble GeoExplorer Global Positioning System (GPS). If any of the 44 baseline saltcedars had resprouted, measurement data were collected for those trees. Decisions on which parameters to measure were based on data collected in the original study (Siegle and Hosler 2010) and on the nature in which saltcedar resprouts. Parameters used in the original study that appeared to be the most valuable in determining effects from the beetle were applied as well as parameters that captured resprouting conditions (e.g. density and stem count). Data collection was streamlined in 2008 to discontinue some measurements. The parameters listed below were measured throughout the study unless otherwise noted. All parameters were the same as those collected in the East and West Plots, although among some variables there were slight differences in collection methods. Statistical analysis was not carried out due to the small sample size (n=13).

Figure 3.—Saltcedar mulching in the Resprout Plot, July, 2006; Pueblo, CO.

Saltcedar Density

Saltcedar plants within the 1 ha plot were flagged and counted (Figure 4). Individual saltcedar stems were counted unless a number of stems were obviously sprouting from one main stem, in which case the cluster was considered one plant.

Number of *D.e.deserticola*

The number of beetle adults, larvae, and egg bundles on the entire resprouted saltcedar was determined. These numbers were estimated within categories of 0, 1-10, 11-50, 51-100, and >100.

Tissue Damage

The percentage of foliage that was damaged by the beetle was estimated through 2007. Dead, shriveled foliage - caused by girdling of branches - was attributed to effects from the beetle. In 2008, only occurrence of beetle damage was noted, not percentage estimates.

Green Foliage

The percentage of foliage on the resprout that was green (i.e. not dead or senescing) was estimated.

Wood without Foliage

The percentage of the resprouted saltcedar that had no foliage was estimated. Wood without foliage was determined by identifying the amount of wood on each tree that did not appear to have foliage from the current year.

Figure 4.—Flagging and counting saltcedar in the Resprout Plot; Pueblo, CO.

Tree volume

The height and diameter of green plant material on each resprouted saltcedar was measured. These values were multiplied to calculate the cross area. Height was determined by measuring the tallest point where the meter rod intercepted live vegetation. Diameter was measured by passing a meter rod through the tree from north to south and from east to west and recording the distance between where the live plant material intercepted the rod at each end.

Vegetation cover

The percent of vegetative ground cover beneath the canopy of each resprouted saltcedar was estimated. This parameter was measured to determine what vegetation, if any, occupied the site following saltcedar removal in the interest of wildlife habitat.

Stem Count

Through 2007, the number of live stems per resprouted saltcedar was counted. Stem count was not collected in 2008. This parameter was measured based on the observation that a number of saltcedar stems respouted in place of one main stem.

East and West Plots

Saltcedar at these sites were mowed in August of 2004. Mechanical treatment involved using a tractor to mow saltcedar at ground level. For those saltcedar that were too large to mow, a chainsaw was used for removal. Prior to treatment, the stand consisted of mid-age saltcedar shrubs or trees. TSC established two plots within these treated sites (East and West Plots as shown in Figure 2) and monitoring began in June of 2005. Originally, the East and West plots were 25m^2 in size. In 2007, the plots were enlarged to 35 m^2. GPS was used to record 1) the location, and 2) measurement data for every saltcedar that had resprouted within each plot. Data collection was streamlined in 2008 to discontinue some measurements. The following parameters were measured throughout the study unless otherwise noted.

Saltcedar Density

Data was collected for every saltcedar plant within each plot, which converted to the number of saltcedar/m^2. Individual saltcedar stems were measured unless a number of stems were obviously sprouting from one main stem, in which case the cluster was considered one plant.

Number of *D.e.deserticola*

The number of beetle adults, larvae, and egg bundles on the entire saltcedar was determined. These numbers were estimated within categories of 0, 1-25, 26-75, 76-100, and >100.

Tissue Damage

The percentage of foliage that was damaged by the beetle was estimated within categories 0, 1-10, 11-50, 51-95, and 96-100 through 2007. Dead, shriveled foliage - caused by girdling of branches - was attributed to effects from the beetle.

Green Foliage

The percentage of foliage on the plant that was green (i.e. not dead or senescing) was estimated within categories 0, 1-10, 11-25, 26-50, 51-75, 76-100 through 2007. In 2008, values were estimated to the nearest 5 percent.

Wood without Foliage

The percentage of the tree that had no foliage was estimated. Wood without foliage was determined by identifying the amount of wood on each tree that did not appear to have foliage from the current year. Through 2007, ranges were used that included 0, 1-25, 26-50, 51-75, and 76-100. In 2008, values were estimated to the nearest 5 percent.

Tree Volume

The height and diameter of green plant material on each saltcedar was measured. These values were multiplied to calculate the cross area. Height was determined by measuring the tallest point where the meter rod intercepted live vegetation. Diameter was measured by passing a meter rod through the tree from north to south and from east to west and recording the distance between where the live plant material intercepted the rod at each end.

Vegetative Ground Cover

The percent of vegetative ground cover beneath the canopy of each saltcedar was estimated. Ground cover was categorized as 0-50 or 50-100. This parameter was measured to determine what vegetation, if any, occupied the site following saltcedar removal in the interest of wildlife habitat.

Stem Count

Through 2007, the number of live stems per resprouted saltcedar was counted. Stem count was not collected in 2008.

Statistical Analysis

Statistical analysis examined beetle damage, green foliage, wood without foliage, tree volume, and vegetative ground cover over time. Analyses included comparisons between consecutive years of the study (2005 vs. 2006, 2006 vs. 2007, 2007 vs. 2008) and comparisons of Year 1 (2005) to Year 4 (2008). The Mann-Whitney nonparametric test was used as data was not normally distributed within any of the variables tested. Pearson correlations were performed using all parameters as well as cumulative annual precipitation.

Both study types – the Resprout Plot and the East and West Plots – are summarized in Table 1.

Table 1. Summary of Resprout Plot and East and West Plot studies.

	Resprout Plot	**East and West Plots**
Study Objectives	1) Potential for saltcedar resprouting following mechanical treatment 2) Assess if health of saltcedar pre-treatment influenced resprouting and herbivory 3) Observe beetle feeding preferences 4) Effects of *D.e.deserticola* herbivory on saltcedar following mechanical treatment	1) Effects of *D.e.deserticola* herbivory on saltcedar following mechanical treatment 2) Observe beetle feeding preferences
Treatment History	Mulched July 2006	Mowed August 2004
Years of Monitoring	Pre-treatment 2004 & 2005 Post-treatment 2006 - 2008	Post-treatment 2005 – 2008
Parameters Measured:		
Saltcedar Density	X	X
# *D.e.deserticola*	X	X
% Tissue Damage	X	X
% Green Foliage	X	X
%Wood w/o Foliage	X	X
Tree Volume	X	X
% Vegetative Ground Cover	X	X
Stem Count	X	X

Results and Discussion

Resprout Plot

The resprout plot was monitored prior to saltcedar removal in October 2004 and in August 2005. Following removal, the plot was monitored in August 2006, June and August 2007, and June and September 2008. Figure 5 shows photos of the Resprout Plot during mulching in July 2006 and

Figure 5.—Resprout Plot during mulching in July 2006 (above) and during monitoring in June 2007 (below); Pueblo, CO.

during monitoring in June 2007. Photos of the 44 trees before and after mulching are shown in Appendix B. Of the 44 identified saltcedar trees, 13 (or 30 percent) had sprouted back by September 2008.

Saltcedar Density

Saltcedar density per the 1 ha plot –as counted in the August/September data set - was on a decreasing trend from 2006 to 2008 (Figure 6). Approximately 426 saltcedar resprouted following mulching in July of 2006 but that number dropped to 259 over the monitoring period. It is unknown whether this decrease was an effect from the beetle or from some other factor such as competition for resources leading to natural thinning of the stand.

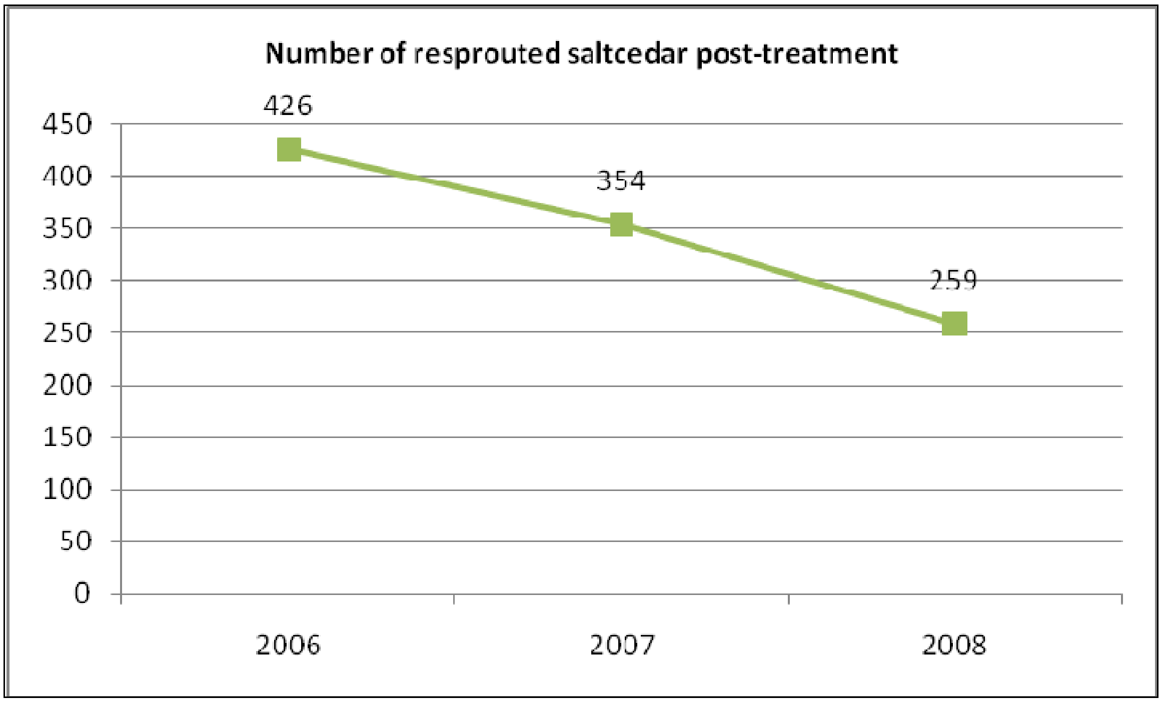
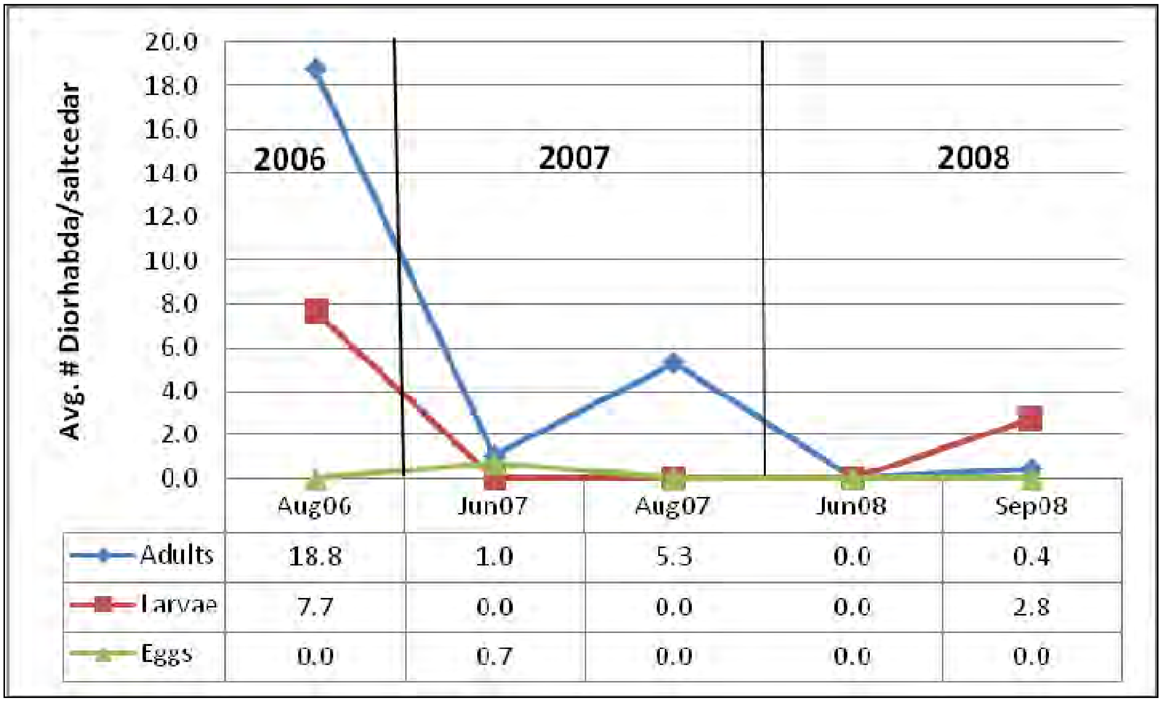


Figure 6.—Number of resprouted saltcedar following treatment from 2006 to 2008 in the Resprout Plot; Pueblo, CO.

Number of *D.e.deserticola*

Estimates of the number of beetle adults, larvae, and egg bundles occurring on each saltcedar began post-treatment in 2006. Presence of the beetle was confirmed during all data collection visits with the exception of June 2008, when no evidence of beetles was detected in the Resprout Plot.

There was no distinguishable pattern in beetle numbers, although more adults were detected in the late summer data set (Figure 7). Trends in beetle numbers more closely followed the East Plot, which was approximately 100 m from the Resprout Plot and closer in proximity than the West Plot. Egg bundles were rarely found during data collection visits in the Resprout Plot. In general, the number of beetles decreased over time, with very few adults and no eggs detected in



	Aug06	Jun07	Aug07	Jun08	Sep08
Adults	18.8	1.0	5.3	0.0	0.4
Larvae	7.7	0.0	0.0	0.0	2.8
Eggs	0.0	0.7	0.0	0.0	0.0

Figure 7.—Average number of *D.e.deserticola* adults, larvae, and egg bundles per saltcedar in the Resprout Plot following treatment from 2006 to 2008, Pueblo, CO.

2008. Beetle population data was only collected two times during the breeding season and due to the limited data set, possible trends in beetle numbers may have gone undetected.

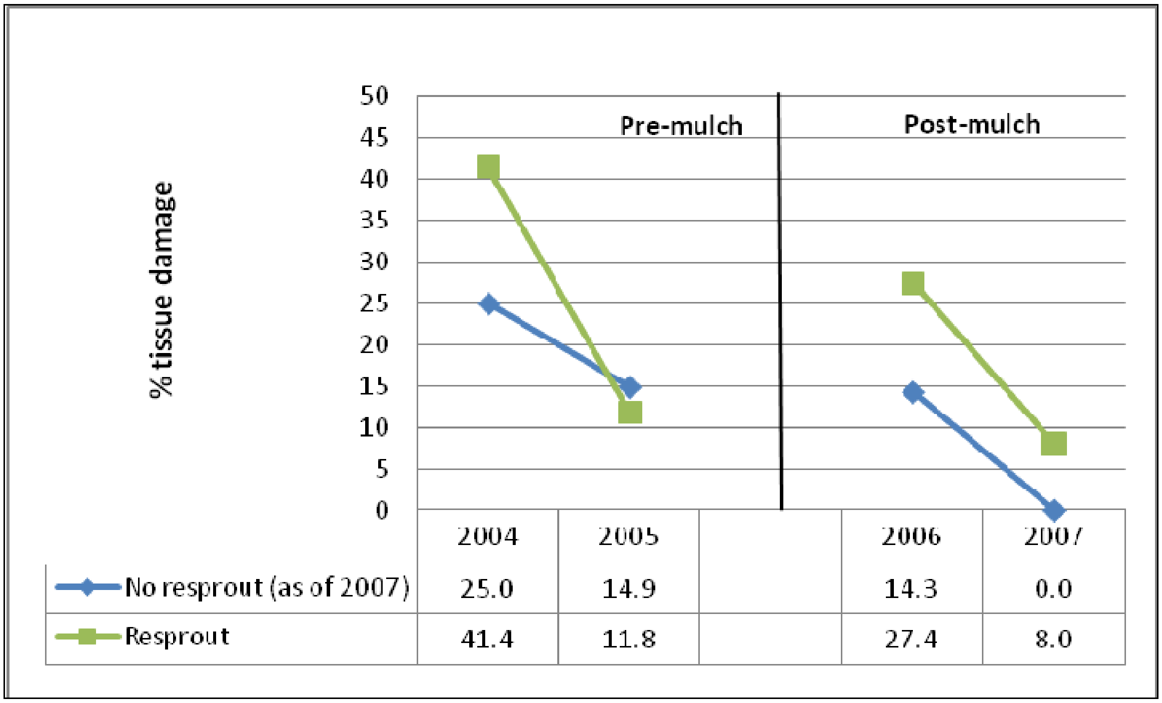
Tissue Damage

The average percent of foliar damage to saltcedar caused by the beetle was not measured in 2008. The amount of tissue damage as measured in August from 2004 to 2007 did not appear to affect the ability of saltcedar to resprout post-treatment since damage was documented at relatively high rates in 2004 (pre-treatment) and 2006 (post-treatment) on those saltcedar that sustained through 2007 (Figure 8). Note that in the figure beetle damage values are documented for the 2006 “no resprout” saltcedar; these values represent saltcedars that had resprouted in 2006 but were no longer alive in 2007.

Green Foliage

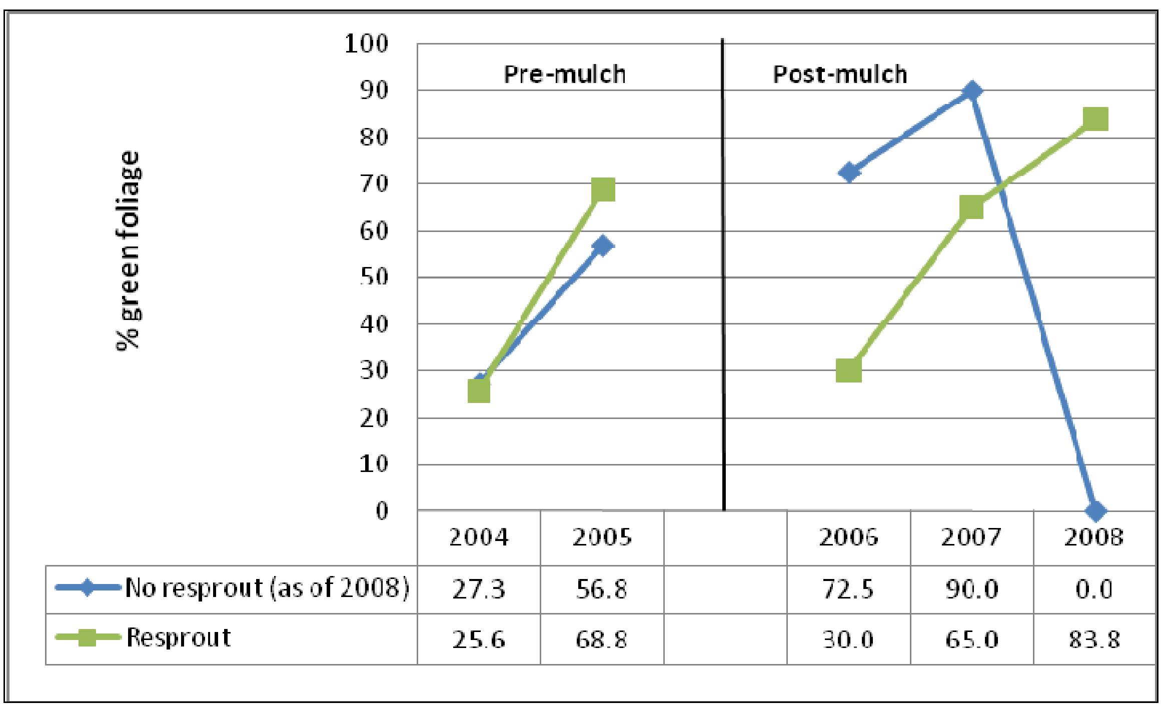
The amount of green foliage as measured in August was not markedly higher prior to mulching in those saltcedar that resprouted after treatment (Figure 9). Consequently, this variable did not appear to affect the ability of saltcedar to resprout. Note that green foliage values are documented for the 2006 and 2007 “no resprout” saltcedar in Figure 9; these values represent saltcedars that had resprouted in 2006 and 2007 but were no longer alive in 2008. The average percentage of green foliage was actually higher following treatment in those saltcedar that did not survive until 2008, although there were only a few saltcedar that were alive in 2006 and 2007 and died by 2008. Therefore the sample size used to calculate this average was very small.

The beetle did not appear to impact the health of surviving saltcedar since the amount of green foliage per saltcedar was on an increasing trend following mulching. The increasing trend,



	2004	2005		2006	2007
No resprout (as of 2007)	25.0	14.9		14.3	0.0
Resprout	41.4	11.8		27.4	8.0

Figure 8.—Average percentage of beetle damage for saltcedar that resprouted and for saltcedar that did not resprout before and after treatment 2004 to 2007; Pueblo, CO.



	2004	2005		2006	2007	2008
No resprout (as of 2008)	27.3	56.8		72.5	90.0	0.0
Resprout	25.6	68.8		30.0	65.0	83.8

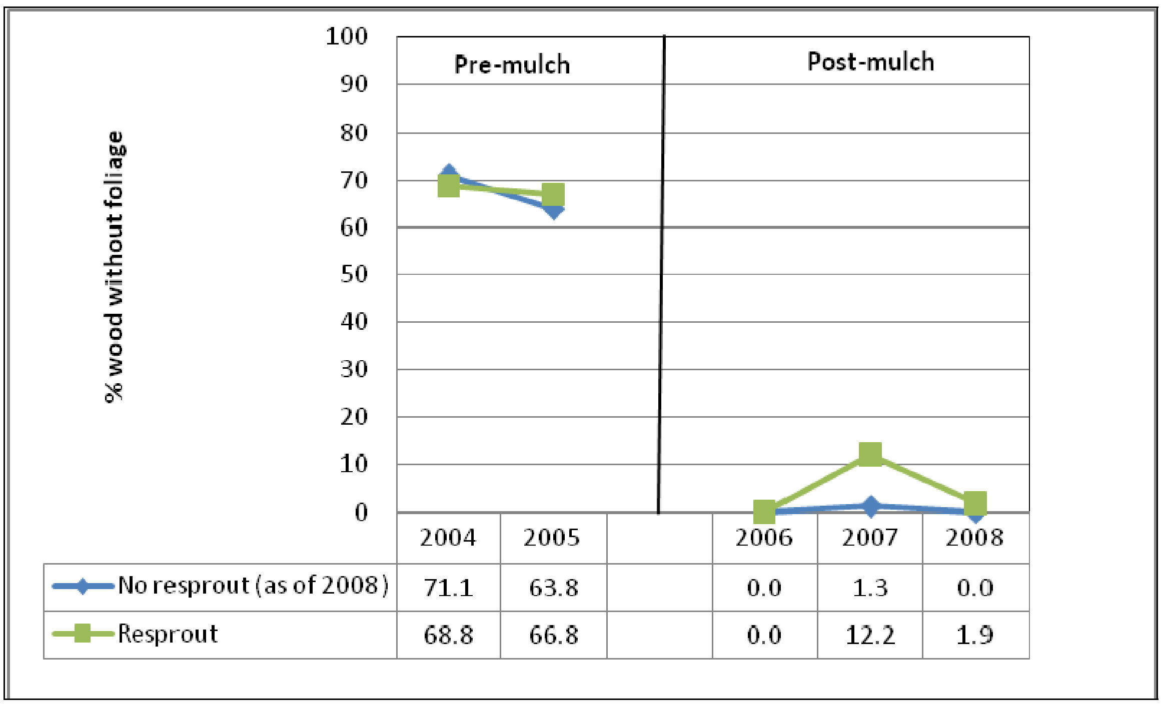
Figure 9.—Average percentage of green foliage for saltcedar that resprouted and for saltcedar that did not resprout before and after treatment 2004 to 2008; Pueblo, CO.

however, may have been related to low numbers of beetles in the resprout plot during the last few years of monitoring.

Wood without Foliage

The average percentage of wood without foliage slightly decreased during pre-mulching years in both saltcedar that resprouted and in those that did not resprout after mulching (Figure 10). Note that in the figure wood without foliage values are documented for the 2006 and 2007 “no resprout” saltcedar; these values represent saltcedars that had resprouted in 2006 and 2007 but were no longer alive in 2008.

Post-mulch, there was no wood without foliage documented until the second year when values were higher among those saltcedar that survived throughout the study. There does not appear to be any evidence that the percentage of wood without foliage affected the ability of saltcedar to resprout after mulching, nor did there appear to be an impact from the beetle on this variable as the amount of wood without foliage decreased in 2008. Again, this result may have been related to low numbers of beetles detected during the last few years of monitoring.

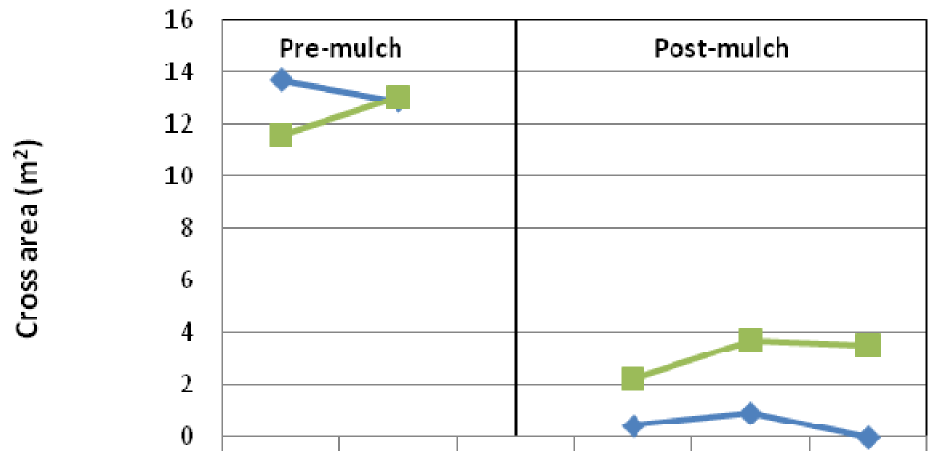


	2004	2005		2006	2007	2008
No resprout (as of 2008)	71.1	63.8		0.0	1.3	0.0
Resprout	68.8	66.8		0.0	12.2	1.9

Figure 10.—Average percentage of wood without foliage for saltcedar that resprouted and for saltcedar that did not resprout before and after treatment 2004 to 2008; Pueblo, CO.

Tree Volume

Figure 11 shows the average cross area per saltcedar for trees that resprouted after mulching treatment and for those that did not resprout as of September 2008. Note that cross area values are documented for the 2006 and 2007 “no resprout” saltcedar in Figure 11; these values represent saltcedars that had resprouted in 2006 and 2007 but were no longer alive in 2008.



	2004	2005		2006	2007	2008
No resprout (as of 2008)	13.7	12.9		0.4	0.9	0.0
Resprout	11.6	13.0		2.2	3.7	3.5

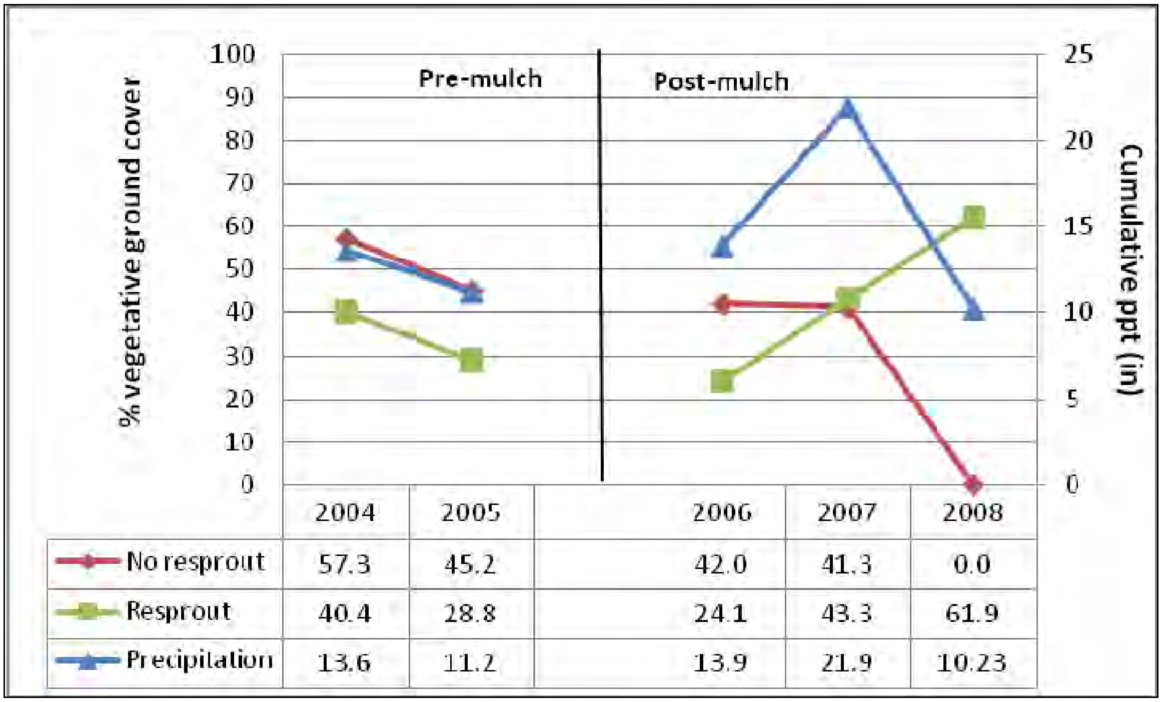
Figure 11.—Average cross area for saltcedar that resprouted and for saltcedar that did not resprout before and after treatment 2004 to 2008; Pueblo, CO.

There was an increase of 1.4 m in cross area before treatment in those saltcedar that resprouted after treatment and a decrease of 0.8 m in cross area of those that did not resprout. There may have been some correlation with the general health of the plant before mulching - as expressed by an increasing or decreasing volume – and the likelihood of it resprouting after mulching. The smaller size of saltcedar that resprouted in 2006 and 2007 but did not survive until 2008 may indicate less vigor in plants that eventually died. In any event, beetles did not appear to impact development of saltcedar that survived over the course of the study. It is difficult to determine if the lack of growth in 2008 was an effect from the biocontrol agent since beetle numbers were relatively low during the last two years of monitoring.

Vegetative Ground Cover

The average percentage of vegetative ground cover beneath the canopy of resprouted saltcedar in August and cumulative annual precipitation is graphed in Figure 12. Prior to mulching, vegetative ground cover decreased between 2004 and 2005, as did annual precipitation. After mulching, vegetative ground cover increased with time under saltcedar that resprouted, from 24.1 percent in 2006 to 61.9 percent in 2008. There did not appear to be an association with precipitation post-mulching, at least based on results in 2008.

These results suggest that vegetation beneath resprouted saltcedar became more established over time and was not heavily affected by shading from the overstory. Variables that affect shading, such as the amount and type of foliage, did not appear to associate with the percentage of ground cover. As damaged foliage and wood without foliage decreased and green foliage increased (presumably leading to an increase in the amount of shading), vegetative ground cover increased. At any rate, saltcedar were probably not large enough to cause significant shading and the



	2004	2005	2006	2007	2008
No resprout	57.3	45.2	42.0	41.3	0.0
Resprout	40.4	28.8	24.1	43.3	61.9
Precipitation	13.6	11.2	13.9	21.9	10.23

Figure 12.—Average percent of vegetative ground cover beneath the canopy for saltcedar that resprouted and for saltcedar that did not resprout before and after treatment and cumulative annual precipitation 2004 to 2008; Pueblo, CO.

understory continued to spread. Kochia (*Kochia scoparia*), an introduced forb, was observed to be the dominant plant species growing beneath resprouted saltcedar within the Resprout Plot.

Stem Count

The average number of stems per saltcedar pre- and post-mulching is shown in Figure 13.

The average number of stems per saltcedar increased from 5 prior to mulching (an average of 2004 and 2005 values) to 10 after mulching (an average of 2006 and 2007 values) among resprouted trees. The number of stems per saltcedar was not measured in 2008. These results indicated that saltcedar removal by mulching at this site caused an increase in the number of stems through resprouting.

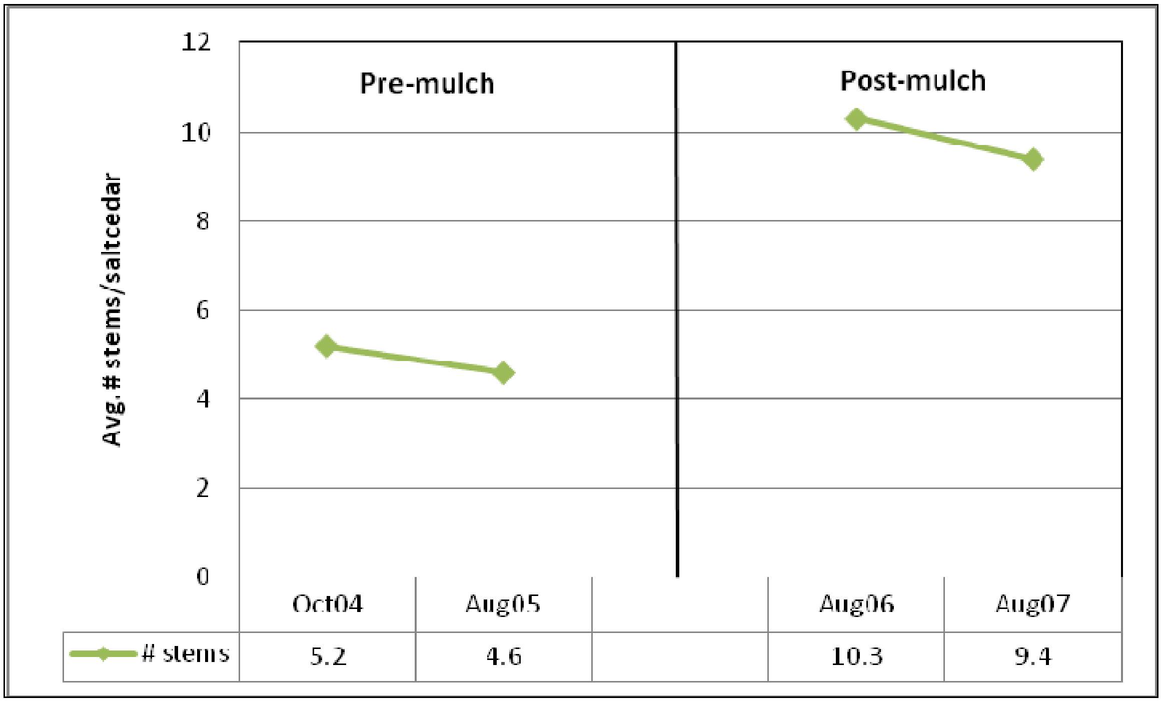


Figure 13.—Average number of saltcedar stems that resprouted before and after treatment 2004 to 2007; Pueblo, CO.

East and West Plots

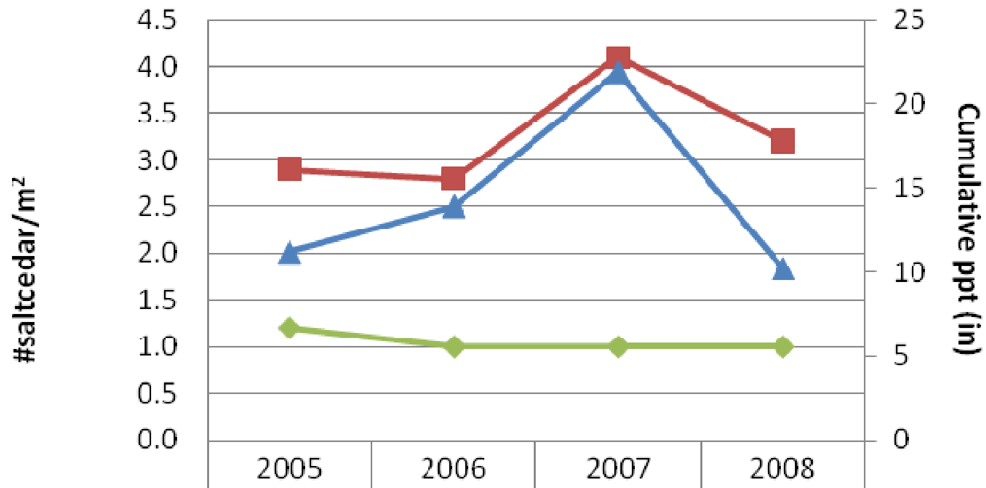
East and West Plots were monitored in early June and late August of each year from 2005 to 2008. Photos taken within each plot are shown in Figure 14. Correlation coefficients (r), sample sizes, and P-values are listed in Appendix A for both plots. The strength of correlation analysis was limited by a relatively small sample size (n= 3 or 4) for percent tissue damage, cumulative annual precipitation, and saltcedar density because there was only one value for each of these parameters per year.

Saltcedar Density

Saltcedar density (an average of the two data sets for each year) decreased slightly from 1.2/m^2 to 1.0/m^2 in the East Plot and increased from 2.9/m^2 to 3.2/m^2 in the West Plot from 2005 to 2008 (Figure 15). The West Plot was adjacent to a pond, and the increased soil moisture at that site was likely a factor in the high number of resprouted saltcedar. The density of saltcedar within the West Plot appeared to be associated with cumulative precipitation (Figure 6), though there was not a statistically significant correlation (r=0.8427, P=0.157). An association seems logical since precipitation would have affected the water level of the pond, which in turn would have influenced the amount of water available to saltcedar resprouts at the West Plot.

In general, the beetle did not appear to have a sizeable impact on the regeneration of saltcedar post-treatment. The biocontrol agent may have reduced the spread of saltcedar, however, particularly in the East Plot where density remained the same from 2006 to 2008. Nevertheless,

Figure 14.—Saltcedar within the East (above) and West (below) plots, July 2007; Pueblo, CO



	2005	2006	2007	2008
East Transect	1.2	1.0	1.0	1.0
West Transect	2.9	2.8	4.1	3.2
Precipitation	11.2	13.9	21.9	10.2

Figure 15.—Saltcedar density in the East and West Plots and cumulative precipitation per year from 2005 to 2008, Pueblo, CO.

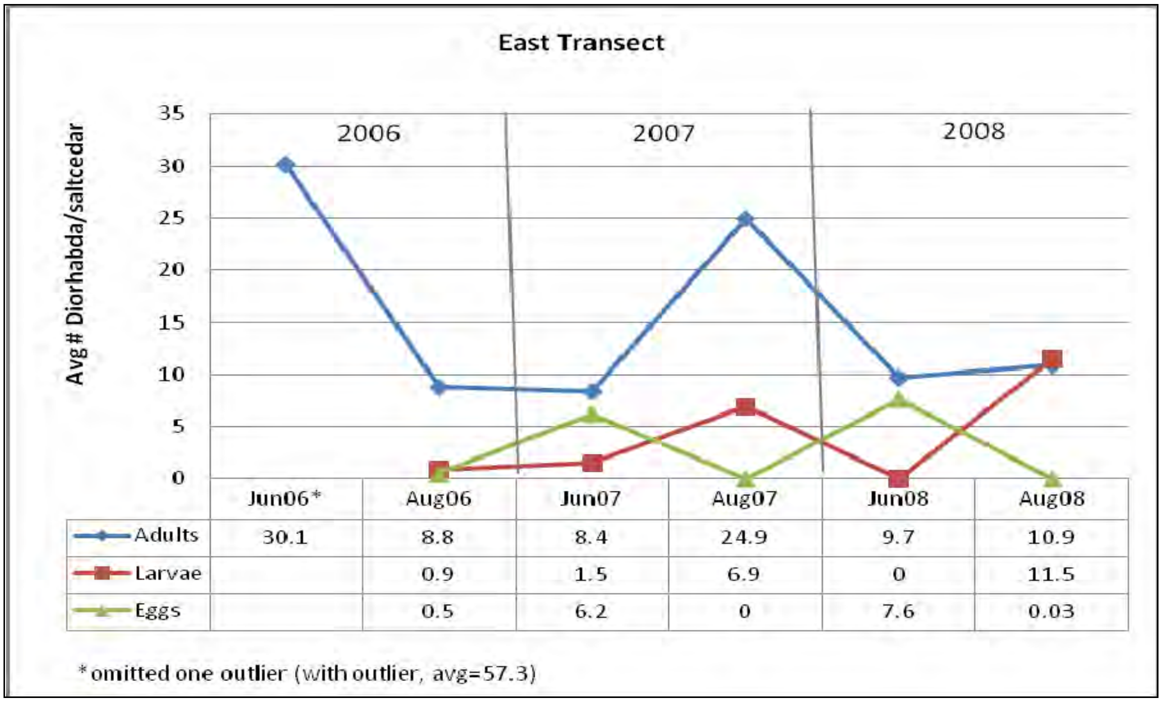
it is impossible to determine if saltcedar would have increased at this plot in the absence of the beetle.

Number of *D.e.deserticola*

Presence of the beetle was confirmed during all data collection visits with the exception of June 2005, when no beetles were detected in the West Plot. In 2005, only the presence or absence of beetles was recorded. In the East Plot, some form of the beetle (i.e. adults, larvae, or egg bundles) was detected on 71 percent of saltcedar in June of 2005 and on 85 percent of the saltcedar in August of 2005. In the West Plot, as mentioned there were no beetles detected in June of 2005, and 5 percent of trees had beetle detections in August of 2005. Estimates of the number of beetle adults, larvae, and egg bundles occurring on each saltcedar began in 2006, although in June of 2006, only adult beetles were counted.

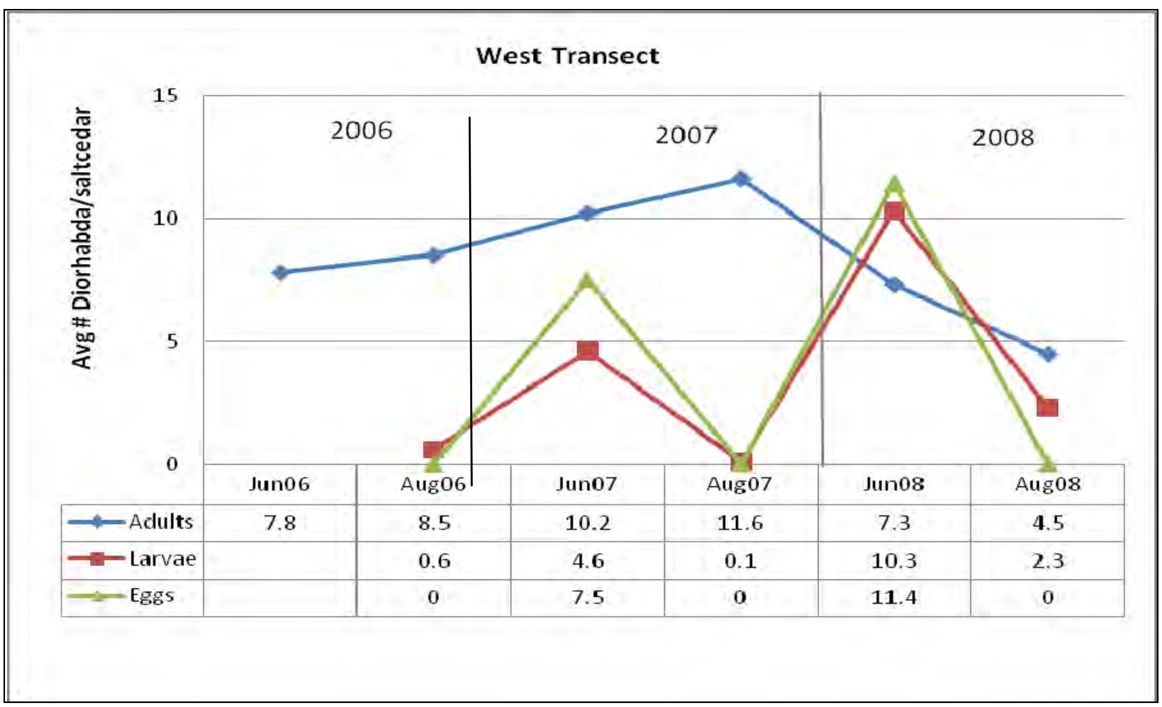
There were no distinguishable patterns in beetle numbers among plots and years (Figures 16 and 17). Typically, there were more adults detected in the August/September data sets. More larvae were detected in the August dataset in the East Plot, while more larvae were detected in the June dataset in the West Plot. Egg bundles were found in higher numbers in June than in August in both plots.

A rough approximation of the beetle's lifecycle at the Pueblo site begins with overwintering adults emerging about the beginning of May and laying eggs approximately 10 days later. The



	Jun06*	Aug06	Jun07	Aug07	Jun08	Aug08
Adults	30.1	8.8	8.4	24.9	9.7	10.9
Larvae		0.9	1.5	6.9	0	11.5
Eggs		0.5	6.2	0	7.6	0.03

Figure 16.—Average number of *D.e.deserticola* adults, larvae, and egg bundles per saltcedar in the East Plot from 2005 to 2008, Pueblo, CO.



	Jun06	Aug06	Jun07	Aug07	Jun08	Aug08
Adults	7.8	8.5	10.2	11.6	7.3	4.5
Larvae		0.6	4.6	0.1	10.3	2.3
Eggs		0	7.5	0	11.4	0

Figure 17.—Average number of *D.e.deserticola* adults, larvae, and egg bundles per saltcedar in the West Plot from 2005 to 2008, Pueblo, CO.

first generation of adult beetles emerged around early July and also produced eggs. The second generation of adults emerged late in August and fed for a few weeks before overwintering by the end of September (Eberts et al. 2005).

There was evidence that another generation of beetles developed during the breeding season over the study period. The beetles originally evolved in higher latitudes with longer day lengths than in Pueblo, and early in the study there were fewer generations than in the native environment. After a few years the beetles seemed to adapt to the shorter day lengths and did not go into reproductive diapause until later in the season, allowing them to produce another generation (O'Meara, per.com.). Three periods of egg laying were completed, with the second generation completing their lifecycle and dying and a third generation overwintering (Eberts, per.com.).

Some trends in beetle numbers observed in each plot could be explained by the beetle's lifecyle. In most instances (including the Resprout Plot), there were more adults detected later in the summer, which was consistent with a generation of adults just emerging in late August. A higher number of egg bundles in early June coincides with the first generation of adults laying eggs in mid to late May. Interestingly, the two plots did not always show the same patterns in beetle numbers even though the sites were only about 600 m apart. The variability that was observed is difficult to explain with data from only 2 site visits per breeding season.

Tissue Damage

The average percentage of foliar damage by the beetle in August increased considerably from 2005 to 2006 in both plots – from 11.1 to 87.6 percent in the East Plot and from 5.7 to 97.6 percent in the West Plot (Figure 18). In 2007, beetle damage decreased from the previous year to 55.1 percent in the East Plot but stayed essentially the same as 2006 at 97.2 percent in the West Plot. The average percent of tissue damage per saltcedar was approximated for 2008 using percent green foliage and general observation since actual values were not collected this year. The amount of foliar damage from the beetle decreased during the final year of monitoring in both plots.

In both plots, percentage of damage appeared to be somewhat associated with the adult beetle population (Figure 18), however a statistically significant correlation was only found in the West Plot ($r=0.9593$, $P=0.041$). Damage was lowest in 2005, when saltcedar were just beginning to sprout back after mowing in fall of 2004 and there was probably not a prolific spread of beetles into the area from the original stand yet. The relatively high amount of tissue damage observed in 2006 and 2007 correlated with higher numbers of adult beetles detected within plots during those years. The number of adult beetles decreased in 2008, along with the amount of damage caused. A more apparent association between damage and the number of larvae would have been expected since larval herbivory causes the most amount of foliar damage due to girdling of the branches at this life stage.

Green Foliage

The average percentage of green foliage per resprouted saltcedar as measured in August of each year was closely linked to the percentage of beetle damage (Figure 19). This was supported by strong correlations in both the East Plot ($r= -0.992$, $P=0.008$) and the West Plot ($r= -0.999$, $P<0.000$). As would be expected, the percentage of green foliage showed an inverse relationship

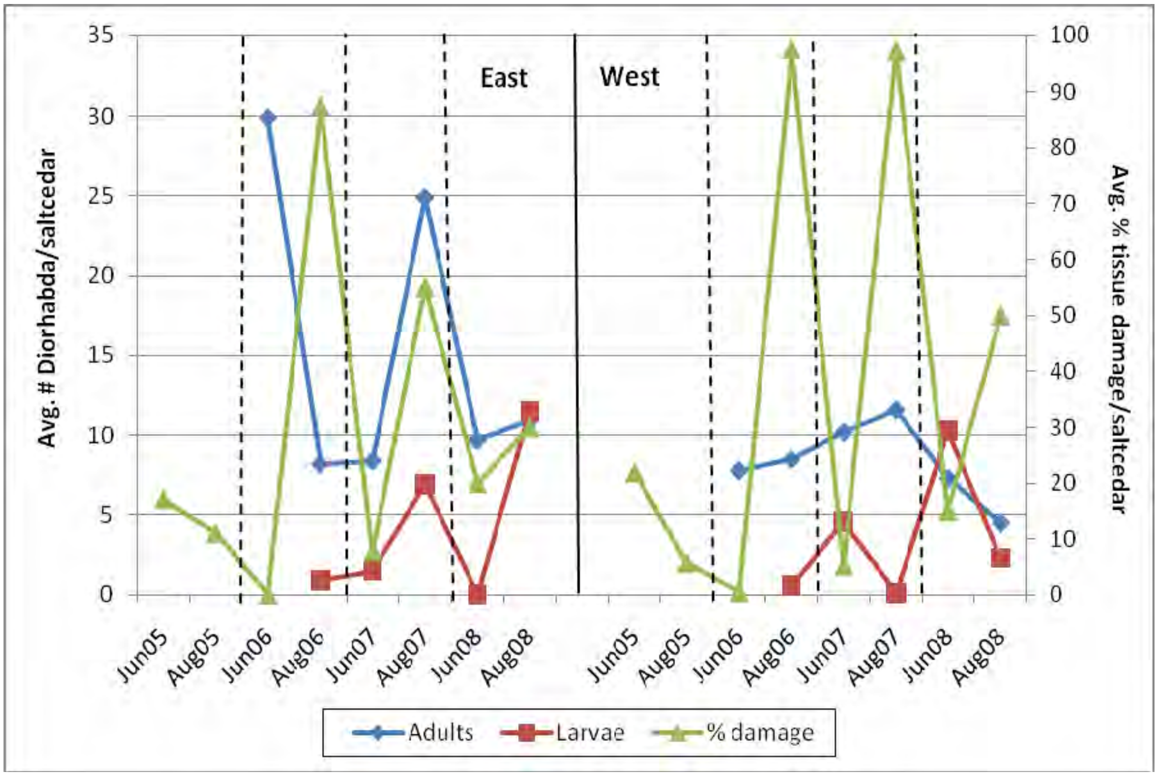
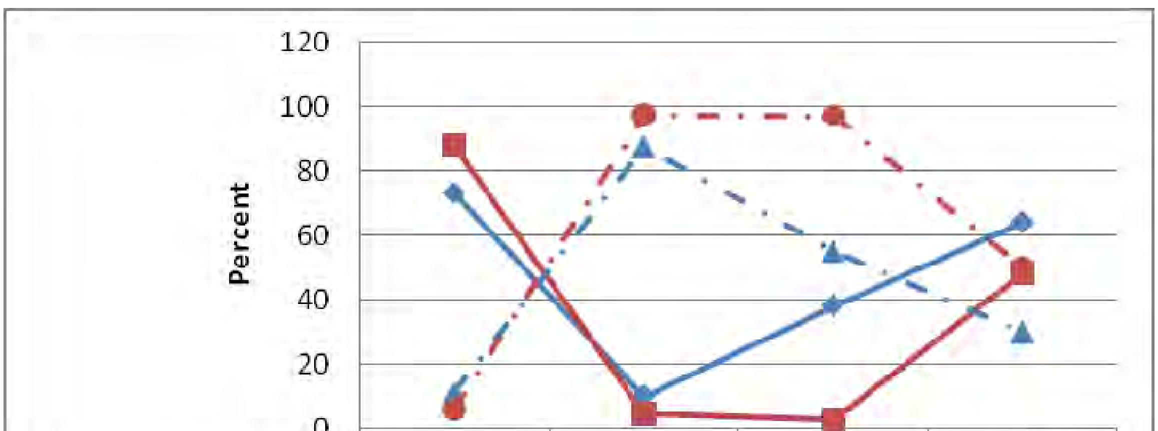


Figure 18.—Average percentage of beetle damage and the average number of *D.e.deserticola* adults and larvae per resprouted saltcedar in datasets from 2005 to 2008 in the East and West Plots, Pueblo, CO.



	2005	2006	2007	2008
East % Green	73.2	9.9	38.1	64.0
West % Green	88.0	4.6	2.7	48.4
East % Damage	11.1	87.6	55.1	30.0
West % Damage	5.7	97.6	97.2	50.0

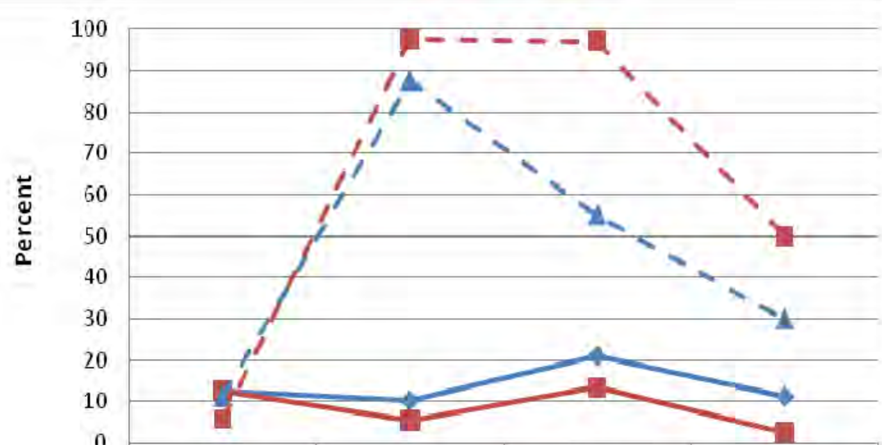
Figure 19.—Average percentage of green foliage and of tissue damaged by the beetle per resprouted saltcedar in August in the East and West Plots from 2005 to 2008, Pueblo, CO.

to the percentage of foliar damage in both plots. That is, when the amount of green foliage was highest in 2005, the amount of foliar damage was lowest (Figure 19) and vice versa in 2006 and 2007. There was also a statistically significant correlation between the number of adult beetles and the percentage of green foliage (r= –0.3840, P=0.000), but this inverse relationship was only significant in the West Plot. The average percentage of green foliage increased in both plots in 2008 – almost to levels of 2005 in the East plot – while the average percentage of foliage damaged by the beetle decreased. Results indicated that although the beetle did affect resprouted saltcedar foliage at the plot sites, the impact was not sustaining.

Wood without Foliage

The average percentage of wood without foliage in August in the East Plot decreased from 12.4 percent in 2005 to 10.2 percent in 2006, increased again to 21 percent in 2007 and finally decreased to 11.2 percent in 2008, for a negligible decrease over the monitoring period (Figure 20). In the West Plot, percentage of wood without foliage also decreased from 2005 to 2006 – from 12.6 to 5.4 percent – then increased to 13.4 percent in 2007 and finally decreased to a low of 2.6 percent, for a total decrease of 10.0 percent over the monitoring period.

There wasn't an obvious correlation between beetle damage and wood without foliage (Figure 20). The relatively high percentage of damage in 2006 may have led to the increase in the percentage of wood without foliage the following year, but the drop in 2008 indicated this was not a long-term impact.



	2005	2006	2007	2008
East % wood w/out foliage	12.4	10.2	21	11.2
West % wood w/out foliage	12.6	5.4	13.4	2.6
East % damage	11.1	87.6	55.1	30.0
West % damage	5.7	97.6	97.2	50.0

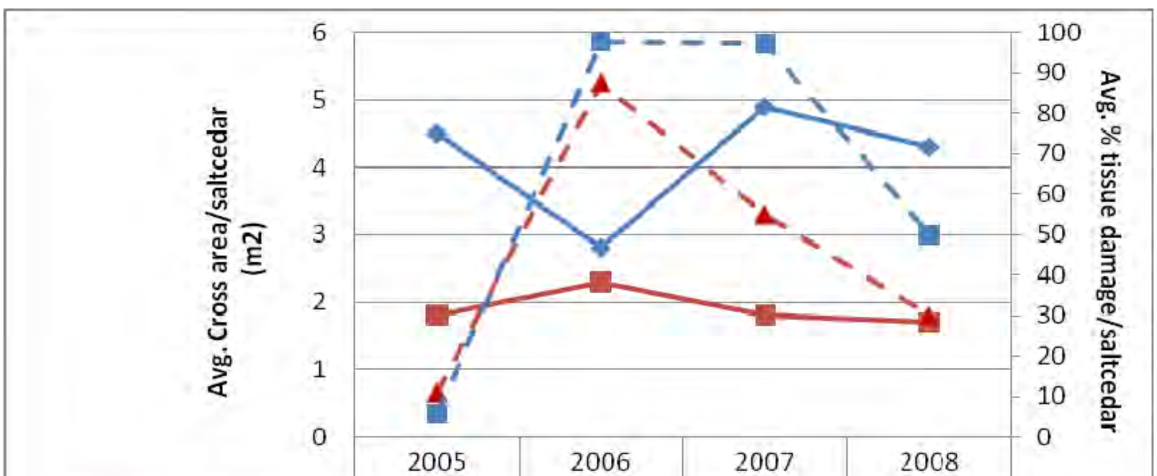
Figure 20.—Average percentage of wood without foliage compared to average percentage of tissue damaged by beetles in August from 2005 to 2008 in the East and West Plots, Pueblo, CO.

The study period may not have been long enough to detect possible impacts from the beetle on wood without foliage, which is a long-term effect relative to other measurements. Estimates of the percentage of wood without foliage were never very high over the course of monitoring, with an average of 21 percent being the most observed.

Tree Volume

The average cross area (height x diameter) of saltcedar as measured in August showed opposite trends in the two plots (Figure 21). In the East Plot, cross area decreased from 4.5 m^2 in 2005 to 2.8 m^2 in 2006 and increased again to 4.9 m^2 in 2007, then fell to 4.3 m^2 in 2008, for a minimal decrease over the monitoring period. In the West Plot, cross area increased slightly from 1.8 m^2 in 2005 to 2.3 m^2 in 2006 and returned to initial levels in 2007 and 2008, resulting in a slight decrease over the monitoring period. The average size of saltcedar was smaller in the West Plot where density was higher.

In the East Plot, the percentage of beetle damage may have affected tree volume – beetle damage was highest in 2006 when cross area was lowest – but this trend was not observed in the West Plot. No significant correlations were found in either plot between the percentage of damage and tree volume. Although there was some variability in the cross area of live vegetation between years and plots, over the course of the monitoring period there was not a noteworthy change. These results suggest that although beetles may have caused a reduction in the rate of growth, they did not prevent the development of saltcedar at either plot.



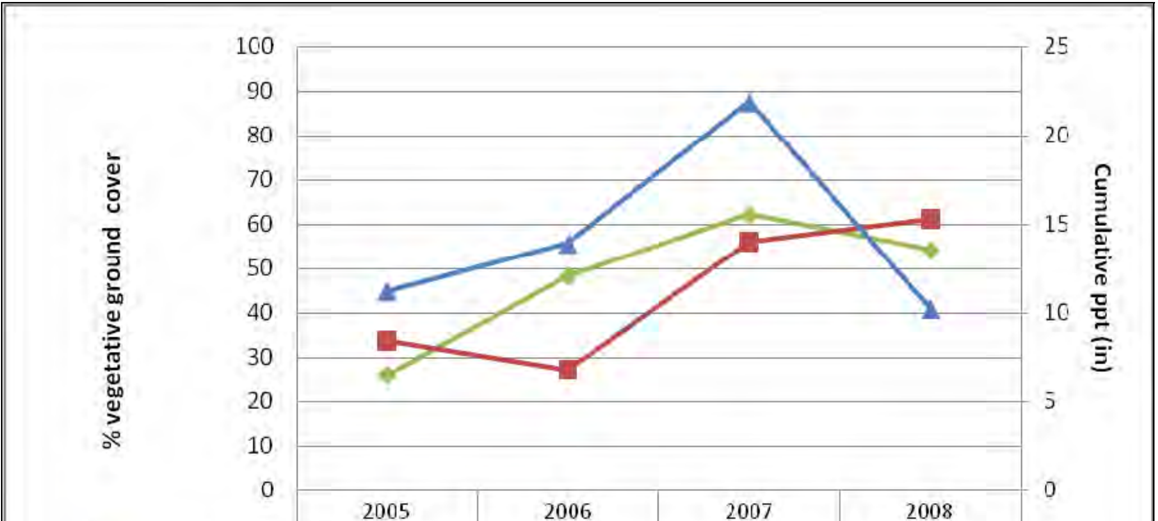
	2005	2006	2007	2008
East cross area	4.5	2.8	4.9	4.3
West cross area	1.8	2.3	1.8	1.7
East % damage	11.1	87.6	55.1	30.0
West % damage	5.7	97.6	97.2	50.0

Figure 21.—Average cross area (height x diameter) compared to average percent tissue damage by beetles per saltcedar in August in the East and West Plots from 2005 to 2008, Pueblo, CO.

Vegetative Ground Cover

The average percentage of vegetative ground cover beneath the canopy of resprouted saltcedar in August is graphed in Figure 22. As with other parameters measured there was variability over the years of monitoring. In general vegetative ground cover increased over time, from 26.0 percent in 2005 to 48.9 percent in 2008 in the East Plot and from 33.8 to 60.2 percent during the same time period in the West Plot.

Vegetation beneath resprouted saltcedar became more established over time and did not appear to be heavily affected by shading from the overstory. The percentage of ground vegetation did appear to be influenced by precipitation, with somewhat of a correlation shown when graphed (Figure 22), though correlations were not statistically significant in either plot. In the West Plot, there also appeared to be an association with tree volume –understory vegetation increased as tree volume decreased – but again this was not a statistically significant correlation and this association was not observed in the East Plot. No other related variables – such as percentage of green, damaged, or no foliage – appeared to influence the amount of vegetative ground cover.



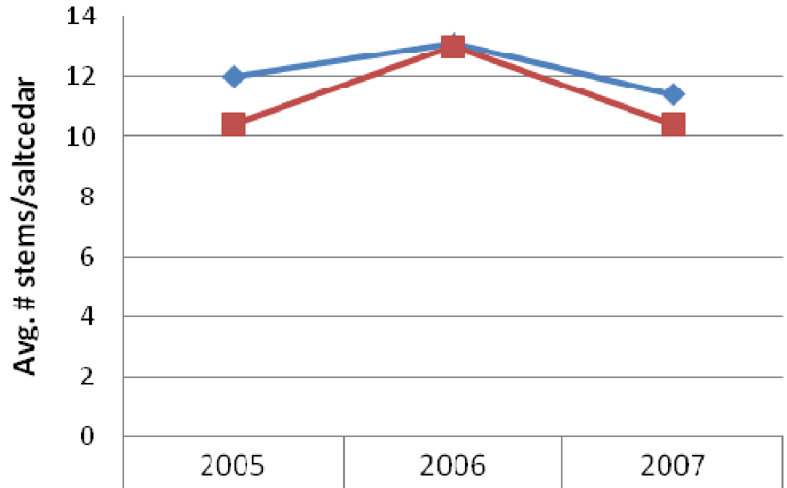
	2005	2006	2007	2008
East Transect	26.0	48.3	62.2	54.2
West Transect	33.8	27.2	56.1	61.2
Precipitation	11.2	13.9	21.9	10.2

Figure 22.—Average vegetative ground cover beneath the canopy of resprouted saltcedar in August in the East and West Plots and cumulative annual precipitation from 2005 to 2008, Pueblo, CO.

Stem Count

The average number of stems per saltcedar post-treatment is shown in Figure 23.

The average number of stems per resprouted saltcedar did not change substantially post-treatment. The number of stems per saltcedar was not measured in 2008. Stem numbers were



	2005	2006	2007
East Transect	12.0	13.1	11.4
West Transect	10.4	13.0	10.4

Figure 23.—Average number of stems per resprouted saltcedar in August in the East and West Plots from 2005 to 2008; Pueblo, CO.

just slightly higher than in the Resprout Plot post-treatment, where the average number of stems per plant were 10.3 in 2006 and 9.4 in 2007. There is no pre- treatment data for the East and West Plots, therefore it is unknown if stems increased after mowing.

Statistical Analysis

Statistical analysis comparing consecutive years and the first and last year of monitoring for selected variables provided few conclusive results. Although there were a number of significant differences found between consecutive years among variables, data were erratic and no consistent trends were observed in either plot (Table 2).

When examining data over the entire monitoring period (i.e. first year vs. last year), only two variables showed significant changes over time in the East Plot. The percentage of tissue damage caused by the beetle significantly increased from 11.1 percent in 2005 to 52.3 percent in 2007. The percentage of vegetative ground cover also significantly increased from 26.0 percent to 48.9 percent from 2005 to 2008 (Figure 22). Both of these variables were on a decreasing trend the last year of monitoring, therefore outcomes may have changed if the study continued.

In the West Plot, statistical comparisons between first and last years of the study showed significant changes in all variables except tree volume. As in the East Plot, both the percentage of tissue damage and vegetative ground cover significantly increased over the monitoring period. Tissue damage increased from 5.7 percent in 2005 to 97.2 percent in 2007 and vegetative ground cover increased from 33.8 percent in 2005 to 60.2 percent in 2008 (Figure 22). Similar to the East Plot, the average percentage of tissue damage was on a decreasing trend in 2008.

Table 2.—Statistical results for selected variables comparing consecutive years and the first and last year of the study within the East and West Plots using the Mann-Whitney test of medians; Pueblo, CO. Alpha = 0.05.

Plot	Years	Tissue damage*	Green foliage	Wood w/out foliage	Tree Volume	Vegetative ground cover
East	2005 vs 2006	2005<2006 P<0.001	2005>2006 P<0.001	2005>2006 P=0.032	2005>2006 P=0.012	2005<2006 P<0.001
	2006 vs 2007	2006>2007 P<0.001	2006<2007 P<0.001	2006<2007 P<0.001	2006<2007 P=0.040	2006<2007 P=0.020
	2007 vs 2008	NA	2007<2008 P<0.001	2007>2008 P=0.006	2007=2008 P=0.403	2007>2008 P=0.005
	First vs Last	2005<2007 P<0.001	2005=2008 P=0.138	2005=2008 P=0.898	2005=2008 P=0.819	2005<2008 P<0.001
West	2005 vs 2006	2005<2006 P=0.0	2005>2006 P=0.0	2005>2006 P=0.0	2005<2006 P=0.029	2005>2006 P=0.015
	2006 vs 2007	2006=2007 P=0.825	2006>2007 P<0.001	2006<2007 P=0.0	2006>2007 P=0.008	2006<2007 P=0.0
	2007 vs 2008	NA	2007<2008 P=0.0	2007>2008 P=0.0	2007=2008 P=0.329	2007=2008 P=0.342
	First vs Last	2005<2007 P=0.0	2005>2008 P=0.0	2005>2008 P=0.0	2005=2008 P=0.255	2005<2008 P=0.0

*No data collected in 2008, note that First vs Last is 2005 vs 2007
Highlighted boxes = significant difference at the 95 percent confidence level.

The average percentage of green foliage and of wood without foliage significantly decreased over the study period in the West Plot. The average percentage of green foliage decreased from 88.0 percent in 2005 to 48.4 percent in 2008 (Figure 19). The average percentage of wood without foliage decreased from 12.6 percent to 2.6 percent over the same period (Figure 20). In both plots in 2008, the percentage of green foliage was on an increasing trend and wood without foliage was on a decreasing trend, indicating that the beetle did not appear to have a long-term impact on the health of saltcedar foliage post-treatment

Conclusions

Resprout Plot

Only 30 percent of the original 44 trees resprouted in the Resprout Plot, and from 2005 to 2007 the average number of individual stems per saltcedar doubled from 5 to 10. The number of saltcedar within the entire hectare plot decreased from 426 to 259 from 2006 to 2008, which differed from the East and West Plots where density either increased or decreased only slightly. This result may have been linked to beetle damage, though the actual reasons for the decrease in the number of saltcedar were unknown. Based on observations by mulching operators in April

2007, saltcedar in the Resprout Plot appeared to exhibit retarded growth compared to other sites they treated where biological control was not used. Due to the nature of this study there were no control plots available for comparison. Control plots with similar climatic and hydrologic conditions would have been located in close proximity to the study area, and therefore subject to herbivory by the beetle. Consequently, it is unknown whether the number and volume of resprouts within the plot were less than they would have been without the presence of the beetle.

One of the objectives of the Resprout Plot study was to examine the rate that saltcedar resprouted following mechanical treatment. Saltcedar is able to resprout vigorously from the root crown, therefore it was somewhat surprising that only 30 percent of the baseline trees had regrown after treatment, although mulching may have been effective at removing root crowns on some trees. The 13 trees that resprouted, however, produced a total of 211 stems (the average of totals from post-treatment years 2006 and 2007), while the 44 baseline trees had a total of 190 stems (the average of totals from pre-treatment years 2004 and 2005). Thus, even though individual trees may not have resprouted at high rates, those that resprouted did so in high numbers, ultimately increasing the number of stems. Mulching (as opposed to mowing) could potentially increase the number of stems that resprout because if the root crown is not completely removed, the meristematic tissue is disrupted. This causes the plant to branch rather than grow from one main stem. In any event, resprouting in this plot was more dispersed than in the mowed East and West Plots, where shoots emerging from one main stem were more obvious.

The likelihood of saltcedar resprouting may have been related to the health of the tree prior to mulching as expressed by an increasing or decreasing cross area. There did not appear to be any correlations or trends in variables that supported an impact on saltcedar resprouts from the biocontrol agent. In general, the number of beetles detected in the Resprout Plot decreased over time, with very few adults, larvae or eggs detected in 2008. In 2007 and 2008, fewer beetles were detected in the Resprout Plot than in the East and West Plots. Whether this difference was related to the method of mechanical treatment or to the position of the plot is unknown.

East and West Plots

The East and West Plots generally exhibited similar trends in all variables except the average number of beetles. Apparently, the plots were located far enough apart that the beetles were at slightly different life stages during each site visit. The variability that was observed in beetle numbers is difficult to explain due to the limited amount of data, which was collected only twice during each breeding season. Our experience indicated that future studies of this nature should include more frequent data collection of beetle numbers and stages. The number of adult beetles detected in both plots when considering both datasets per year was lower in 2008 than in previous years. Beetle numbers were higher in the East and West Plots than in the Resprout Plot in 2007 and 2008, however. Saltcedar density decreased slightly in the East Plot, from 1.2 to 1.0 saltcedar/m^2 from 2005 to 2008. The average number of saltcedar increased in the West Plot, from 2.9 to 3.2 saltcedar/m^2, which was probably due to water availability from an adjacent pond.

With regards to beetle feeding preferences, prior to removal of the original mature stand in spring of 2008, comparisons between the original site and the plots with resprouted foliage were noted. Fewer beetles were observed feeding at the mature site in the spring. Beetles seemed to initially appear in the East and West Plots before being detected in other plots. This may have indicated a preference for younger foliage. Our study did not evaluate whether mechanical treatment (*i.e.* mowing or mulching) actually increased the number of beetles by providing younger, and perhaps more desirable, forage; however it may be worthy of examination in future studies of this nature.

When examining the percentage of beetle damage as it related to other variables, there were no obvious correlations with wood without foliage observed. An association between these two variables could have indicated long-term impacts from beetles. There may have been a slight correlation between tree volume and beetle damage in the East Plot. For example, the average percentage of tissue damage to saltcedar was highest in 2006 when average tree volume was lowest. There was not an obvious link between these two variables in the West Plot. Neither plot showed significant increases in tree volume over the study period, however, which may have indicated that the beetle slowed the growth rate of saltcedar. Also, based on observation, saltcedar cover did not expand in area, which could potentially be attributed to beetle control as well.

These results are not conclusive in determining an effect from the beetle on saltcedar resprouts over the four monitoring periods. Drawing conclusions about the results is difficult since there were no control plots available for comparison. It is unknown whether the number and volume of resprouts within the plots were less than they would have been without the presence of the beetle. The most that can be determined is that saltcedar did not expand in area.

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Siegle, R. and D. Hosler. 2010. Saltcedar Biocontrol at Pueblo, Colorado: Vegetation Monitoring Final Report. Technical Memorandum No. 86-68220-10-03. U.S. Department of the Interior, Bureau of Reclamation, Technical Service Center, Denver, CO.

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PEER REVIEW DOCUMENTATION
PROJECT AND DOCUMENT INFORMATION

Document: Effects of the Biological Control Agent *Diorhabda elongata deserticola* on Resprouted Saltcedar

Date: November 2010 Date Transmitted to Client: November 2010

Team Leader: Denise Hosler

Document Author(s)/Preparer(s): Rebecca Siegle, Denise Hosler

REVIEW REQUIREMENT

Part A: Document Does Not Require Peer Review

Explain ____________________

Part B: Document Requires Peer Review: SCOPE OF PEER REVIEW
Peer Review restricted to the following Items/Section(s): All

Reviewer(s): Scott O'Meara, Susan Broderick

REVIEW CERTIFICATION

Peer Reviewer - I have reviewed the assigned Items/Section(s) noted for the above document and believe them to be in accordance with the project requirements, standards of the profession, and Reclamation policy.

Reviewer Signature: [signature] Review Date: 10/15/10

Reviewer Signature: [signature] Review Date: 12/16/10

Preparer - I have discussed the above document and review requirements with the Peer Reviewer and believe that this review is completed, and that the document will meet the requirements of the project.

Team Member Signature: [signature] Date: 1/21/2011

Appendix A

Pearson Correlations for the East and West Plots
Correlation coefficients, Samples sizes, P-values

West Plot

	Saltcedar Density	%Damage	% Wood w/out foliage	%Green foliage	%Veg ground cover	Tree volume	Precipitation	Year
#Adults	0.6518 4 0.348	0.9593 4 **0.041**	0.0755 406 0.129	-0.3840 406 **0.000**	0.0272 406 0.585	0.3806 406 **0.000**	0.7625 4 0.238	0.1773 406 **0.000**
Saltcedar Density	↳	0.4376 4 0.562	0.4661 4 0.534	-0.4576 4 0.542	0.7133 4 0.287	-0.4577 4 0.542	0.8427 4 0.157	0.4801 4 0.520
%Damage		↳	-0.1379 4 0.862	-0.9996 4 **0.000**	0.0962 4 0.904	0.5241 4 0.476	0.6809 4 0.319	0.3884 4 0.612
% Wood w/out foliage			↳	-0.0740 406 0.137	-0.0192 406 0.898	-0.219 406 0.700	0.5895 4 0.411	-0.3312 406 **0.000**
%Green foliage				↳	-0.0192 406 0.700	0.0831 406 0.095	-0.7002 4 0.300	-0.2947 406 **0.000**
%Ground cover					↳	-0.0660 406 0.1845	0.2467 4 0.753	0.4544 406 **0.000**
Tree volume						↳	0.0488 4 0.951	-0.0583 406 0.241
Precipitation							↳	0.1217 4 0.878

Correlation
Sample size
P-value
Highlighted value = significant P at alpha = 0.05

East Plot

	Saltcedar Density	%Damage	% Wood w/out foliage	%Green foliage	%Veg ground cover	Tree volume	Precipitation	Year
#Adults	No output*	0.9464 3 0.210	-0.1354 106 0.166	-0.1097 106 0.263	0.2032 106 0.037	0.3880 106 0.000	0.5340 3 0.641	-0.0216 106 0.826
Saltcedar Density	↳	-0.7172 4 0.283	-0.1910 4 0.809	0.6297 4 0.370	-0.9047 4 0.095	0.2490 4 0.751	-0.3898 4 0.610	-0.7746 4 0.225
%Damage		↳	0.0028 4 0.997	-0.9921 4 0.008	0.6144 4 0.386	-0.6764 4 0.324	0.4285 4 0.572	0.1162 4 0.884
% Wood w/out foliage			↳	-0.1541 132 0.078	0.0930 132 0.289	-0.2054 132 0.018	0.8955 4 0.105	0.0369 132 0.675
%Green foliage				↳	-0.1545 132 0.077	0.2061 132 0.018	-0.4501 4 0.550	0.0675 132 0.442
%Ground cover					↳	-0.0450 132 0.609	0.7211 4 0.279	0.3289 132 0.000
Cross area						↳	0.3361 4 0.664	0.0579 132 0.509
Precipitation							↳	0.1217 4 0.878

Correlation
Sample size
P-value
Highlighted value = significant P at alpha = 0.05
*Saltcedar density was the same (*i.e.* 1.0/m^2) from 06-08, the years that adult beetles were counted

Appendix B

Photos of 44 saltcedar in the Resprout Plot
October 2004 (pre-mulch) and June 2007 (post-mulch)

Tree 1

October 2004

June 2007

Tree 2

October 2004

June 2007

Tree 3

October 2004

June 2007

Tree 4

October 2004

June 2007

Tree 5

October 2004

June 2007

Tree 6

October 2004

June 2007

Tree 7

October 2004

June 2007

Tree 8

October 2004

June 2007

Tree 9

October 2004

June 2007

Tree 10

October 2004

June 2007

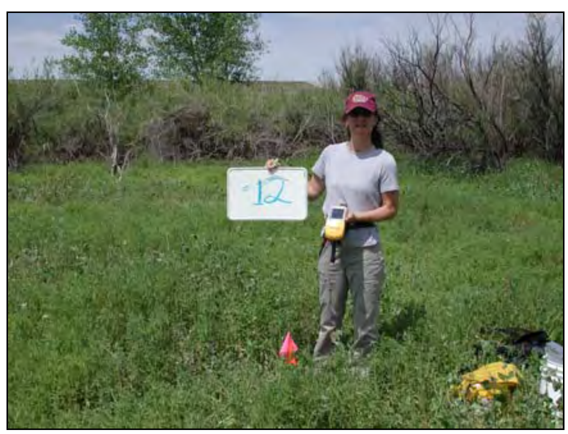
Tree 11

October 2004

June 2007

Tree 12

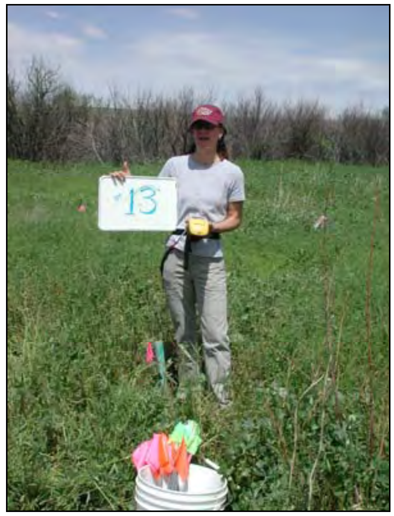
October 2004



June 2007

Tree 13

October 2004



June 2007

Tree 14

October 2004

June 2007

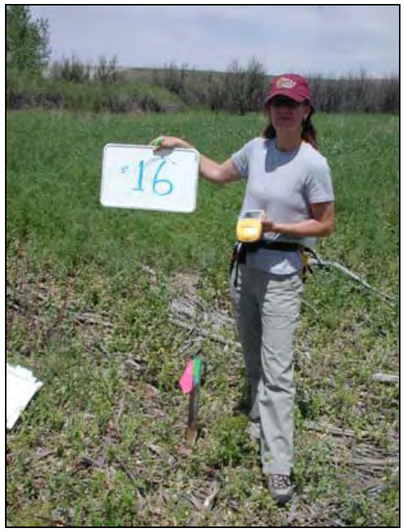
Tree 15

October 2004

June 2007

Tree 16

October 2004



June 2007

Tree 17

October 2004

June 2007

Tree 18

October 2004

June 2007

Tree 19

October 2004

June 2007

Tree 20

October 2004

June 2007

Tree 21

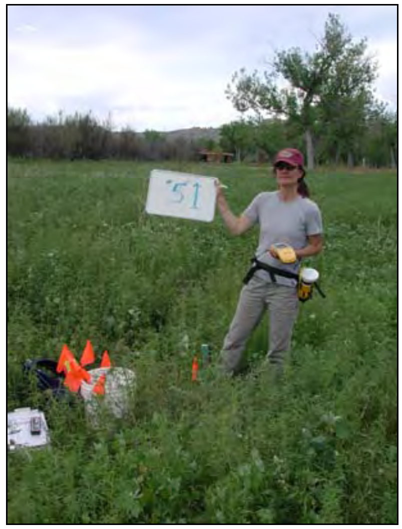
October 2004

June 2007

Tree 22

October 2004

Renumbered to Tree 51



June 2007

Tree 23

October 2004

June 2007

Tree 24

October 2004

June 2007

Tree 25

October 2004

June 2007

Tree 26

October 2004

June 2007

Tree 27

October 2004

June 2007

Tree 28

October 2004

June 2007

Tree 29

October 2004

June 2007

Tree 30

October 2004

June 2007

Tree 31

October 2004

June 2007

Tree 32

October 2004

Tree 33

No photo available

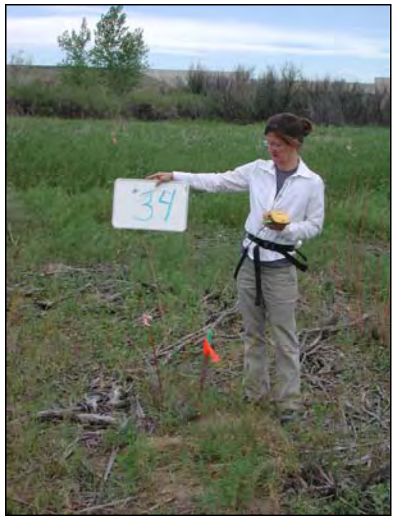
October 2004

Renumbered to Tree 46

June 2007

Tree 34

October 2004



June 2007

Tree 35

October 2004

June 2007

Tree 36

October 2004

June 2007

Tree 37

October 2004

June 2007

Tree 38

October 2004

June 2007

Tree 39

October 2004

June 2007

Tree 40

October 2004

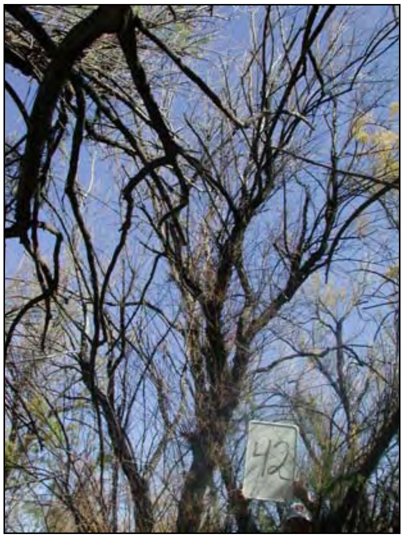
June 2007

Tree 41

October 2004

June 2007

Tree 42



October 2004

June 2007

Tree 43

October 2004

June 2007

Tree 44

October 2004

Renumbered to Tree 52

June 2007