

RECLAMATION

Managing Water in the West

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Saltcedar Biocontrol at Pueblo, Colorado: Vegetation Monitoring Final Report



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

June 2010

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.

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prepared by

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Introduction

Following is a summary of activities related to biocontrol of saltcedar near Pueblo, Colorado as described in Eberts et al. (2005). Research on the biological control of saltcedar (*Tamarix* spp.) began in 1987 when the Bureau of Reclamation (Reclamation) funded initial studies by the U.S. Department of Agriculture's (USDA) Agricultural Research Service (ARS; Deloach 1991). In 1994, permission to release the most promising candidates was requested from USDA Animal and Plant Health Inspection Service. Early in 1995, the U.S. Fish and Wildlife Service listed the southwestern subspecies of the willow flycatcher (*Empidonax traillii extimus*) as a federally endangered species. Because this bird was found to nest in saltcedar in a few areas, permission to release biocontrols was withheld pending investigations into the effects of saltcedar biocontrol on the flycatcher. Once the required National Environmental Policy Act compliance was completed, resulting in a Finding of No Significant Impact (USDA 1999), permits for caged studies and limited field releases followed.

Reclamation and USDA-ARS began studying the biocontrol insects within secure field cages at Pueblo Reservoir in 1997. Pueblo, Colorado was one of the eight proposed sites that were approved for biocontrol release. Permits for field release were received in May of 2001, when the *Diorhabda elongata deserticola* beetles (hereafter beetles) originating in Fukang China were released for biological control. Initial dispersal of the insects in 2001 was limited. As the beetles dispersed, severe defoliation occurred about 1 year after beetles occupied a new area. By 2003, all saltcedar within approximately 40.5 hectares (ha) of the initial release point had been defoliated and in 2004 the insects moved outside of the project area. Some patches of saltcedar trees within the project area were removed in 2004, and again in 2006, as part of a State management effort.

The beetles emerged from overwintering and completed a lifecycle at the Pueblo site each year beginning in 1999 as follows (Eberts et.al. 2003):

- The overwintered adult beetles emerged at the end of April/beginning of May. Saltcedar foliage was beginning to grow at this time, and was at least 2 cm long. These adults layed eggs and died.
- The first generation of new adults emerged at the beginning of July. These adults also layed eggs and died.
- The second generation of new adults emerged mid-to-late August. These adults generally did not lay eggs. They fed for a few weeks and then disappeared into the soil litter for overwintering by the end of September.

There was evidence that another generation of beetles developed during the breeding season over the study period. The beetles 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.).

One of the conditions of release required by the Saltcedar Biological Control Consortium was that monitoring be performed to determine impacts on target and non-target vegetation. Reclamation’s Technical Service Center (TSC) conducted vegetation monitoring from June of 2000 through August of 2007 at the Pueblo site.

The project area comprised approximately 10 hectares at the base of Pueblo Dam, west of the city of Pueblo, Colorado on the Arkansas River (Figure 1). Three separate studies were conducted within the project area. Initial monitoring began in 2000 and included 100 mature saltcedar trees. This sample was reduced to 41 trees in 2003 and is shown in Figure 2 as “Original tree stand”. These trees were selected to assess effects of the beetle on the target species over time. In association with each of the original trees, 1x1 m plots were used to monitor the effects of the beetle on non-target understory vegetation. Saltcedar and Russian olive were mechanically removed from the original tree stand in April of 2008. This original study is the focus of this report.

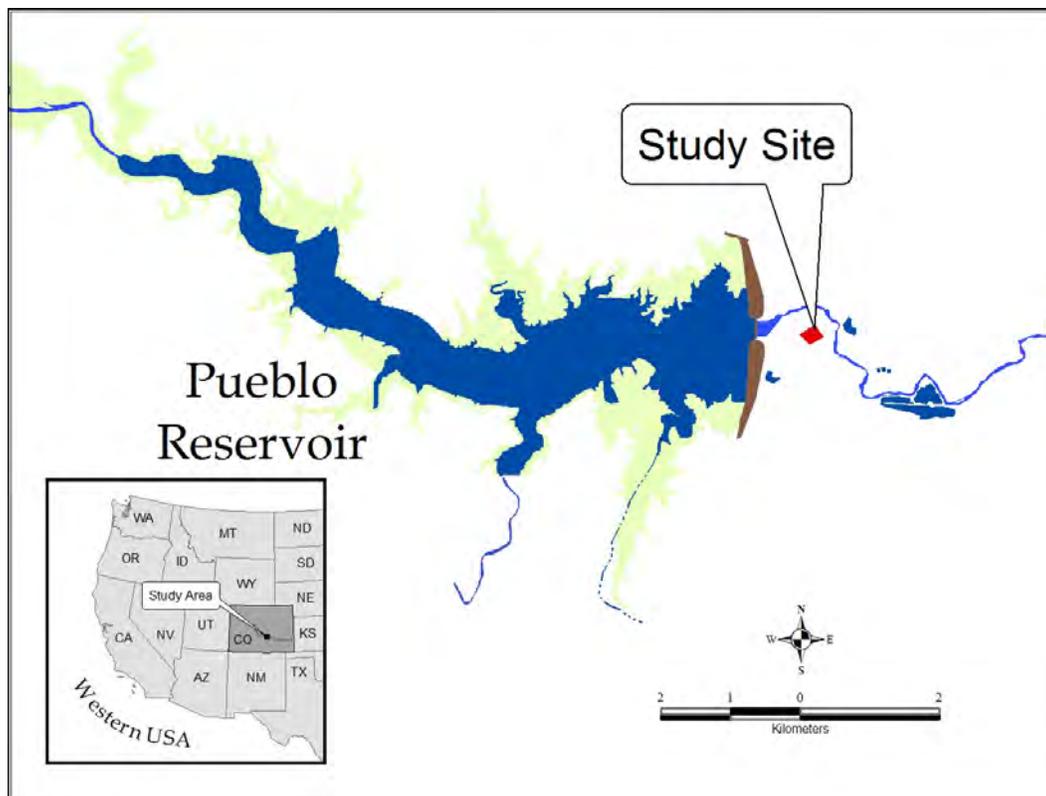


Figure 1.—Location map of saltcedar biocontrol vegetation monitoring site; Pueblo, CO.



In 2004, saltcedar in areas surrounding the original study area were mowed. TSC observed numerous beetles foraging on foliage that had resprouted from the mowed saltcedar, which led to a second study. A monitoring plan using two transects was implemented to examine the potential for the beetle to control saltcedar following mechanical treatment. See East and West transects in Figure 2. Lastly, baseline and post-treatment data were collected at a site that was mulched in 2006. Forty-four mature saltcedar trees were located prior to removal, and the site where each tree had been was located again after treatment to determine resprouting characteristics of saltcedar and the ability of the beetle to control resprouted foliage. See “Resprout plot” in Figure 2. The methods and results of these additional resprout studies are reported in a separate document (Siegle and Hosler 2010).

Methods

Original Tree Stand

One hundred trees were identified and tagged as permanent samples within the project area in 2000 (see Figure 2). Baseline data were collected June through August of that year prior to the beetle release in spring of 2001. From 2001 to 2007, tree measurements were conducted biannually in early June and late August. An intensive methodology was developed by the Saltcedar Biological Control Consortium and was based on standard vegetation monitoring protocol. At the time the sampling protocol was designed, the expected level and timing of damage to saltcedar by the beetles was unknown. As monitoring continued, TSC found that the effects were not great enough during the period of study to warrant such intensive methodology. In 2003, the sample of 100 trees was reduced to 41. In 2005, the sample design was revised to discontinue collection of extraneous data and to simultaneously use field time more efficiently. Forms used to collect saltcedar data are shown in Appendix A. The following parameters were included in data collection throughout the monitoring period.

Number of *D.e.deserticola*

The number of adult and larval beetles on the entire saltcedar was determined. These numbers were estimated within categories of 0, 1-10, 11-100, 101-1000, and >1000. A trial “one-minute count” method for determining the number of beetles on each tree was incorporated in 2005 in which the number of adult and larvae beetles observed within each quarter of the tree during a one minute period were counted and totaled. This method was tested because the upper ranges used in the original protocol for counting beetles exaggerated the number of beetles on the entire tree when values were greater than 10.

Branch Data

The number of individual beetles in each stage (i.e. adult, larvae, egg) was counted on a single branch randomly selected at each cardinal direction of the saltcedar tree. This method was employed to provide an actual value for statistical analysis rather than an estimate.

Tissue Damage

The percentage of damage to foliage caused by the beetle and by *Opsius stactogalus* (a host-specific leafhopper present on site that was introduced to North America with imported saltcedar) was estimated. Senescing foliage in the form of blotchy yellowing of the tree was attributed to effects from the leafhopper. Dead, shriveled foliage - caused by girdling of branches - was attributed to effects from the beetle.

Wood without Foliage

The percentage of the tree that had no foliage was estimated. Wood without foliage was determined by identifying the proportion of total branches on each tree that did not appear to have foliage from the current year.

Foliage Color

The percentage of foliage that was green, senescing/yellow, or dead was estimated for each saltcedar. Estimates for the three categories totaled 100 percent. Factors that may have affected foliage color outside of impacts from insects were not necessarily measured (e.g. season, temperature).

Regrowth

Regrowth referred to live tissue from late season refoliation following herbivory by the beetle. This tissue had an altered, tufted appearance that was easily identifiable from the other foliage. The percentage of foliage that had experienced regrowth was estimated for each tree.

Reproductive status

The presence of flowers was documented, which included all stages of flowering, (ie. buds, open flowers, and seeds) to determine the percentage of reproducing saltcedar.

Tree volume

The height and diameter of live plant material on each saltcedar was measured. These values were multiplied to calculate the cross area. Originally, height was determined by taking one measurement in each quarter of the tree and averaging those values. This method appeared to create a high amount of variability in the data. Therefore the protocol was revised in 2005 and 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.

Plant canopy

A number of methods were used for measuring canopy density and shading over the period of study, including a hand-held densiometer, a light meter, and a light bar. Densiometer readings were collected early in the study and data were inconsistent. A digital light meter was used from 2000 through 2002. Due to the limited amount of data, and because this instrument was used early in the study before significant effects from the beetle would be expected, data was not used. The AccuPAR PAR/LAI Ceptometer light bar was used from 2003 to 2006. It was determined that this instrument provided more precise measurements of canopy cover through the collection of leaf area index (LAI). The LAI of the tree canopy is the area of leaves per unit area of soil surface. The light bar measured the photosynthetically active radiation (PAR), which is a combination of radiation transmitted through the canopy and radiation scattered by leaves within the canopy. PAR radiation is in the 400-700 nanometer waveband, the portion of the spectrum which plants use for photosynthesis. The light bar calculates LAI based on above- and below-canopy PAR readings along with other variables that relate to canopy architecture and the position of the sun. Only data from light bar measurements were used for plant canopy analysis.

Associated woody vegetation

The species and distance from the sample tree of the nearest three neighbors that were woody perennials with a trunk diameter greater than 2.5 cm were collected. This information was collected to monitor any changes in the composition of species of non-target woody vegetation.

Statistical Analysis

Statistical analysis examined wood without foliage, green and dead foliage, reproductive status, tree volume, and plant canopy over time. These variables were statistically compared because they provided data that could be assessed over the long-term and potentially be used as a gauge for effects from the beetle over time. Analyses included comparisons between consecutive years of the study (2000 vs 2001, 2001 vs. 2002...2006 vs. 2007) and comparisons of Year 1 (2000) to Year 8 (2007). The paired t-test was used for normally distributed data and the signed rank nonparametric test was used for data that was not normally distributed. McNemar's test was used for proportional data (i.e. reproductive status).

Pearson correlations and Spearman rank correlations (for non-normal data) were run between variables that included: number of adult and larval beetles, tissue damage, wood without foliage, foliage color, regrowth, reproductive status, tree volume, plant canopy, native grass cover, introduced forb cover, year, and precipitation.

Stepwise regression (Thullen et al. 2008) was used to test for correlations between beetle populations (i.e. adults and larvae in June, August, and total) and climate factors (i.e. cumulative annual precipitation, spring/summer precipitation,

fall/winter precipitation, spring/summer degree days, fall/winter degree days, first freeze, last freeze, maximum winter temperature, minimum annual temperature, and the highest number of consecutive days with temperatures below freezing annually). Each degree day (DD) was calculated using the following equation:

$$DD = (\text{Temp}_{\text{max}} + \text{Temp}_{\text{min}} / 2) - K$$

where Temp_{max} and Temp_{min} were the daily maximum and minimum temperatures and K was the threshold temperature for beetle development. A K of 12.5°C (54.5°F) was used based on estimates from studies conducted with *D.e.deserticola* in 8 locations in the western U.S., including Colorado (Lewis et.al 2003).

Photos

Photographs were taken of each saltcedar for a visual comparison over time.

Associated Understory

In association with each of the saltcedar samples, understory vegetation data was collected. One by one meter (m) quadrats were used to estimate cover of herbaceous and woody plant species in August of each year. Originally, quadrats were permanently located in two positions beneath the tree; one quadrat 0.5 m from the base of the tree, and one quadrat at the dripline of the tree. In 2005, the number of quadrats used was reduced to one at the dripline location. Data collection using 2 quadrats was time-consuming and statistical analysis showed no differences in the data collected between the two quadrats. Therefore, it was determined that there was not an added benefit from having quadrats in two locations, and the sample was reduced to a single quadrat per tree. Forms used to collect understory vegetation data are shown in Appendix A. Cover estimates are described below.

Total Cover

The percent of vegetation cover within 1 m of the ground, the percent of bare soil and the percent of litter cover was estimated for each quadrat, for a total of 100%. The total cover of shrub species above a meter was also estimated as a separate layer.

Relative Cover

The percent relative cover of each of the herbaceous species (including woody seedlings and saplings) present within the quadrat was estimated, for a total of 100%.

Statistical Analysis

The total cover of plant, litter, and bare ground and the relative cover of native grasses and introduced forbs, which were the predominate lifeforms present, were statistically compared over time. Analyses included comparisons between

consecutive years of the study (2000 vs 2001, 2001 vs. 2002...2006 vs. 2007) and comparisons of Year 1 (2000) to Year 8 (2007). The paired t-test was used for normally distributed data and the signed rank nonparametric test was used for data that was not normally distributed. Pearson correlations and Spearman rank correlations (for non-normal data) were run between variables listed under Statistical Analysis in the Original Tree Stand section above.

Photos

Photographs of each of the quadrats were taken to visually document changes in the cover and composition of understory vegetation over time.

Photo Stations

Seven permanent photo stations were established in 2004 and ten more were added in 2005 within the Original Tree Stand project area. The purpose of the photo stations was to visually document changes to saltcedar over time on a landscape level. Locations of the photo stations are shown in Figure 2.

Results and Discussion

Original Tree Stand

In August of 2007, the original 100 saltcedar trees were revisited, which included the 41 trees that remained in the study as well as the 59 trees that had been dropped. Photos were taken and the height and percent of wood without foliage were recorded. Of the original 100 saltcedar trees, 3 had been inadvertently mowed during the State's 2004 saltcedar removal effort. Eight of the trees, or 8 percent, had died.

Statistical analysis comparing data from June 2001 to August 2007 found that the average height of the 100 saltcedars (including only those trees that remained alive throughout the study) significantly increased from 3.9 m in 2001 to 4.6 m in 2007 ($P=0.000$). Therefore it appeared that the beetles did not affect the growth of saltcedar within the study site. The average percentage of wood without foliage (including trees that were both dead and alive at the end of the study) increased significantly from 14.7 percent in 2001 to 47.0 percent in 2007 ($P<0.001$), however, which appeared to show long-term foliar damage from the beetle.

The following results are from data collected from the 41 trees that were measured throughout the study period. Most variables were graphed with *D.e.deserticola* numbers and with the percentage of wood without foliage over

time. The variable “wood without foliage” was chosen for visual comparison because it was a factor that was obviously impacted by beetle herbivory as documented over the study period. Correlation analysis was carried out and correlation coefficients (r), sample sizes, and P-values are shown in Appendix B.

Number of *D.e.deserticola*

Figure 3 shows whole tree estimates for the average number of beetles per tree by dataset (i.e. June and August) from 2000 to 2007. Occasionally, supplemental data were collected outside of this particular study and is included in Figure 4 in an attempt to get a more complete picture of beetle populations over time. The most complete records were collected by TSC in 2003 and 2004, when possible patterns could be detected. Generally, and not surprisingly, higher larval populations followed higher adult populations. In 2003, there appeared to be three generations of adults developing with the first two generations followed by increases in larvae. The third generation from that year apparently overwintered. In 2004, there appeared to be only two generations of adults and larvae.

It is important to note that the number of beetles within the study area changed from week to week as the beetle’s life cycle developed and as adults moved around the site. Therefore the numbers recorded in our data sets, which were only collected consistently for two weeks out of the breeding season and supplemented with random collections, may not have always reflected the actual annual population sizes. At a minimum, data on the estimated number of beetles provided an indication of highs and lows in the population over the study period.

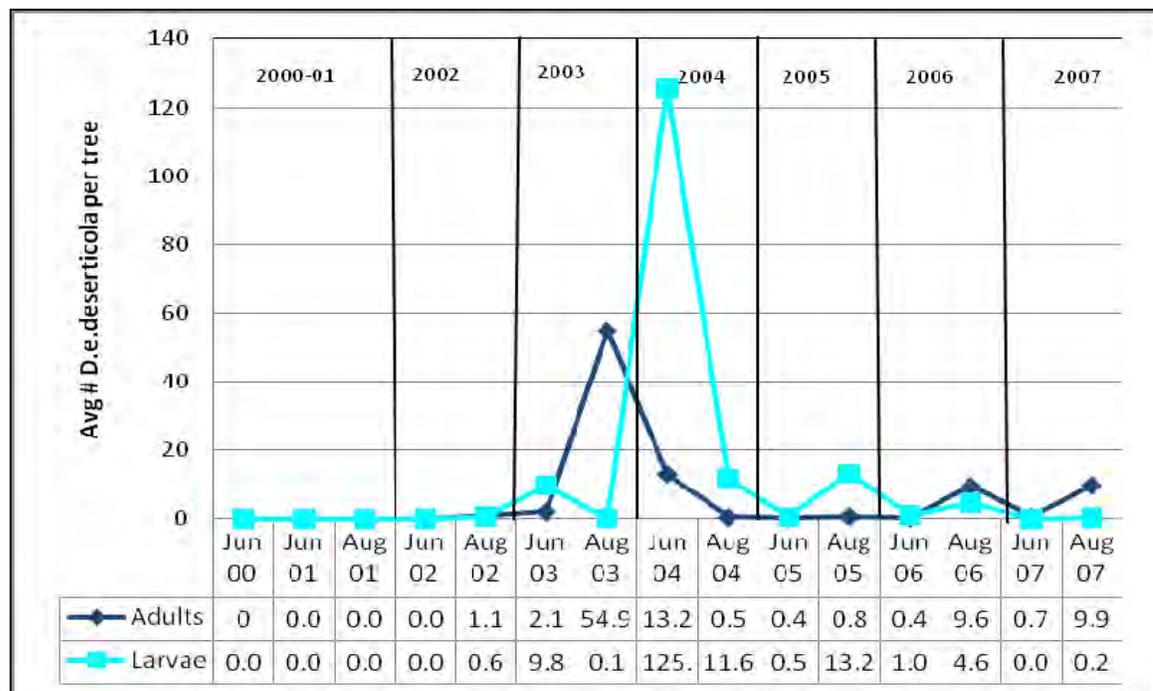


Figure 3.—Average number of *D. e. deserticola* adults and larvae per tree by data set from 2000 to 2007; Pueblo, CO.

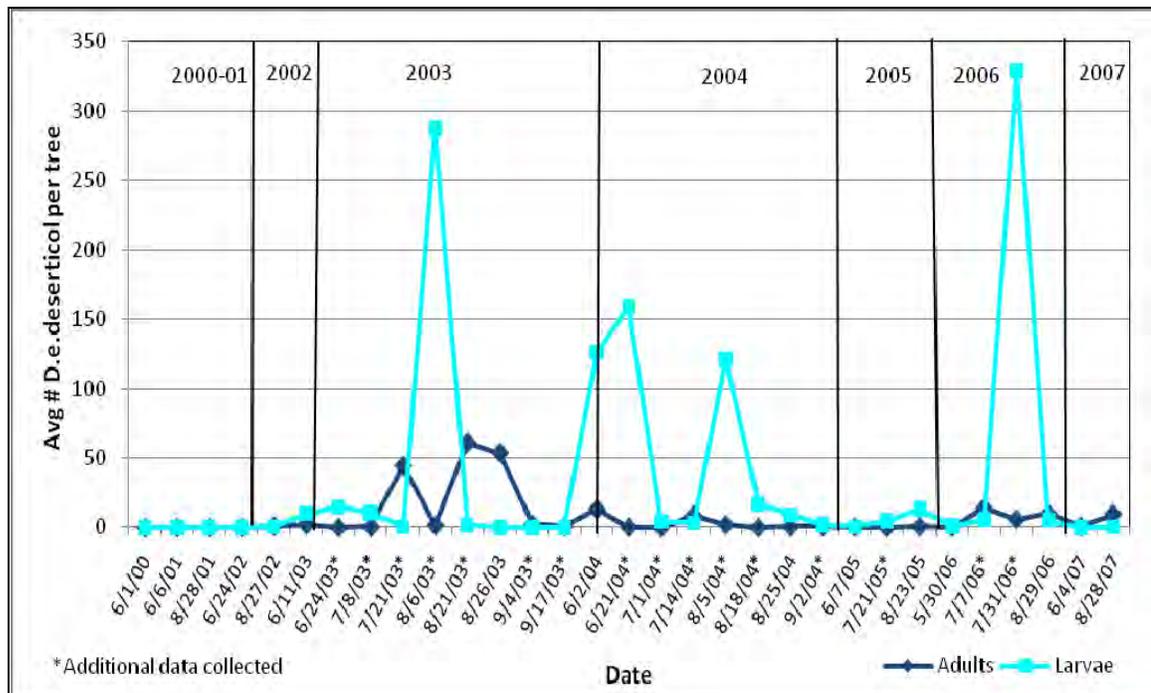


Figure 4.—Average number of *D. e. deserticola* adults and larvae per tree by data set and including supplemental data from 2000 to 2007; Pueblo, CO.

A trial to determine the most effective method for counting beetle populations was conducted from 2005 to 2007. Figure 5 shows differences between the number of beetles estimated with the whole tree count method, which used ranges, and the trial one minute count method, which used actual numbers of beetles counted within a one minute period. When using the midpoint of each range for analysis purposes, values jumped drastically as estimates increased from one range to the next (e.g. 5 versus 55 in the 1-10 and 11-100 ranges, respectively), which caused population estimates to become exponentially higher as values increased. Because not all the beetles present would necessarily be counted in a one minute period, values estimated using the one minute count method could have been potentially lower. Populations were low enough from 2005 to 2007, however, that in most cases all the beetles detected were counted within a minute's time. The one-minute count method therefore appeared to provide a truer representation of the actual number of beetles. As shown in Figure 5, when using the minimum rather than the midpoint of each range to estimate populations using the whole tree count, values were closer to those in the one minute count method, especially as values increased. Statistical analysis found no significant difference between the minimum values of each range used in the whole tree count and the one-minute count values using the signed rank test ($P=0.153$). There was, however, a significant difference between the midpoint values of each range used in the whole tree count and the one-minute count values ($P=0.000$). Therefore, minimum values of each range used in the whole tree count were utilized to estimate beetle populations from 2000-2007.

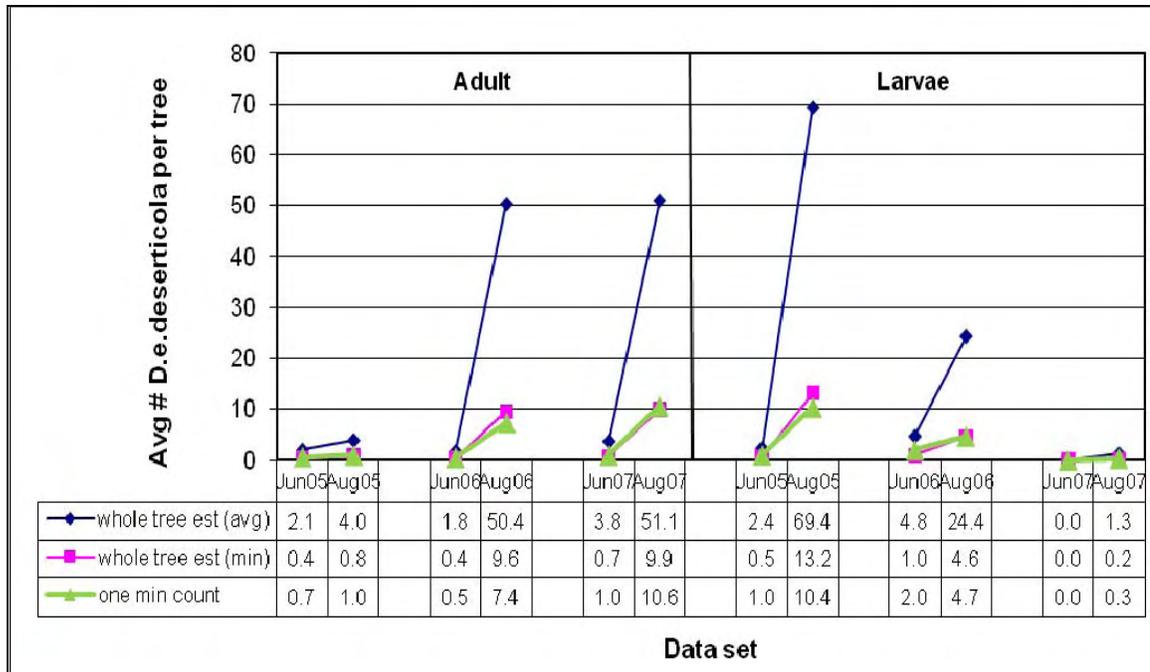


Figure 5.—Comparison of two methods for counting adult and larvae *D. e. deserticola*: whole tree estimates (average and minimum of ranges) and one minute counts; Pueblo, CO.

Table 1 shows climate variables and values that were statistically tested for correlations with beetle numbers. Climate data were collected from a nearby weather station and were not specific to each sample (i.e. tree). Stepwise regression analysis indicated that of the 10 climate variables considered in relation to adult and larval beetle populations, only degree days in October through March explained the variation in mean total larvae ($r=0.7036$, $P=0.037$). These results suggested that warmer temperatures from October to March (when beetles overwinter in soil litter) were related to increased populations of larvae throughout the following breeding season. The same results would be expected for the adult population since presumably adults and larvae were linked

Table 1.—Values for climate variables that were statistically tested for correlations with *D.e.deserticola* adult and larval populations; Pueblo, CO.

Year	Precipt Oct-Mar (in)	Precipt Apr-Sep (in)	Precipt annual (in)	Min temp (degrees F)	Max wint temp (degrees F)	Last freeze (# day in yr)	First freeze (# day in yr)	Consec days of freeze	Avg degree days Oct-Mar	Avg degree days Apr-Sep
2002	1.39	2.49	3.88	-3	80	115	279	46	-14.82	13.92
2003	3.43	5.9	9.33	-12	80	100	289	25	-14.76	12.76
2004	2.09	11.5	13.59	-5	83	122	287	58	-12.78	9.76
2005	3.25	7.91	11.16	-10	75	132	267	27	-14.67	10.86
2006	3.54	10.36	13.9	-8	79	116	272	47	-13.63	13.63
2007	6.15	15.76	21.91	-9	83	116	284	52	-15.73	12.73

throughout the breeding season. However, only June populations of adults were significantly correlated with June populations of larvae ($r=0.9972$, $P<0.001$) and with total larvae ($r=0.9907$, $P<0.001$). See Appendix B for results of Pearson correlations between beetle populations and climate variables. When June adult populations were the dependent factor in stepwise regression, no significant relationships with climate variables were found. The significant correlation does not necessarily verify that warmer temperatures caused higher numbers of larvae. When evaluating the outcome, the small sample size ($n=6$) should be taken into account, which led to a less vigorous statistical analysis.

Branch data

The total number of beetle adults, larvae, and eggs detected on four branches per tree by data set is shown in Figure 6. These values follow a similar pattern to the whole tree counts for adults and larvae, keeping in mind that branch data were summarized using the total number of beetles found on branches on all trees, while the whole tree count was an average number of beetles per tree, so actual numbers vary. The number of adults detected was generally lower than the number of larvae and eggs due to the adult's mobility, which caused them to be much less likely to be detected on a single branch. We found that population estimates from branch data did not provide more specific information than whole tree counts. Branch data did, however, provide egg counts, a variable that was not measured in the other types of data collected.

Earlier in the study, the numbers of beetles detected in each cardinal direction were statistically compared to determine if the beetle showed any preference in

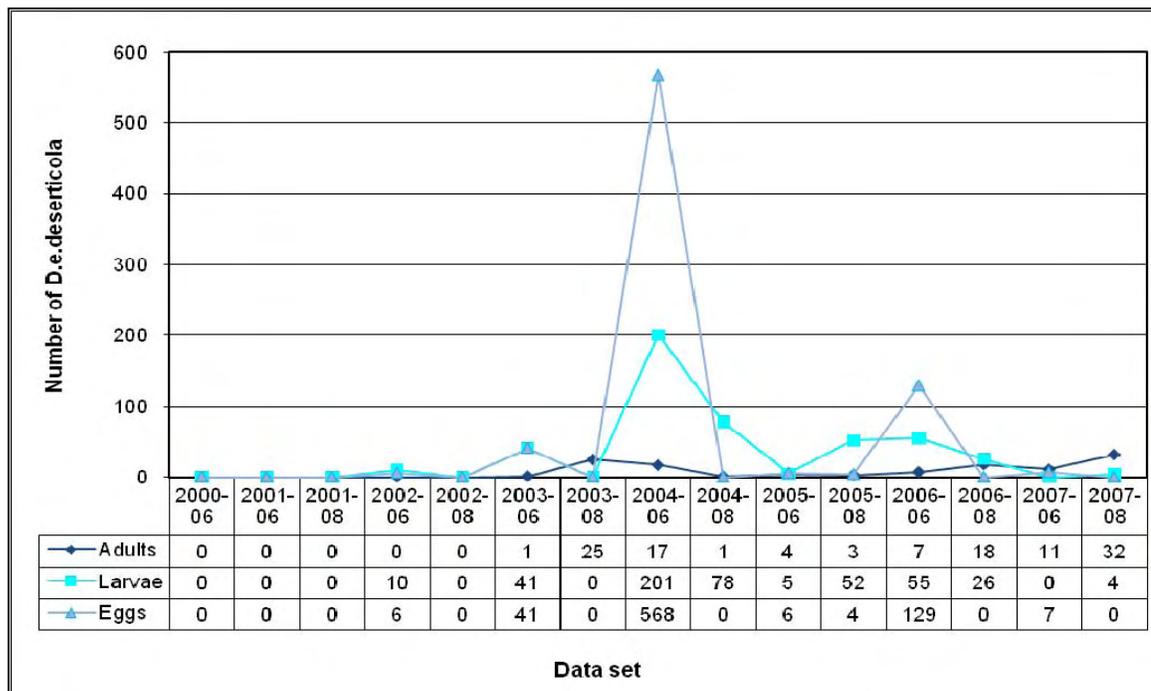


Figure 6.—The total number of *D. e. deserticola* adults, larvae, and eggs detected on four branches per tree by dataset from 2000 to 2007; Pueblo, CO.

location on the tree (Eberts et al, 2005). No statistically significant differences were found between locations and no further analysis was conducted regarding this variable.

Tissue Damage

The percentage of foliar damage to the trees caused by the biocontrol beetle and the leafhopper were estimated and results are shown in Figure 7. The peak damage from the beetle appeared to follow the peak number of beetle larvae by 1-2 weeks within each breeding season. There was also a pattern showing the percentage of damage on the rise when beetle larvae populations were highest. Larval herbivory causes the most amount of foliar damage due to girdling of the branches at this life stage, therefore damage would be expected to increase when the larval populations were highest.

Leaf hopper damage showed a slight correlation with beetle damage based on Figure 7. Generally both types of damage appeared to increase simultaneously. Leafhoppers may have been attracted to beetle-damaged plants, possibly in response to volatile chemical cues released from defoliation. Or a climate favorable to both insects may have increased populations at the same time. It was also possible that beetle damage was often misinterpreted as leafhopper damage.

Significant correlations were found between beetle damage and all variables except reproductive status and precipitation (see Appendix C for all r- and P-

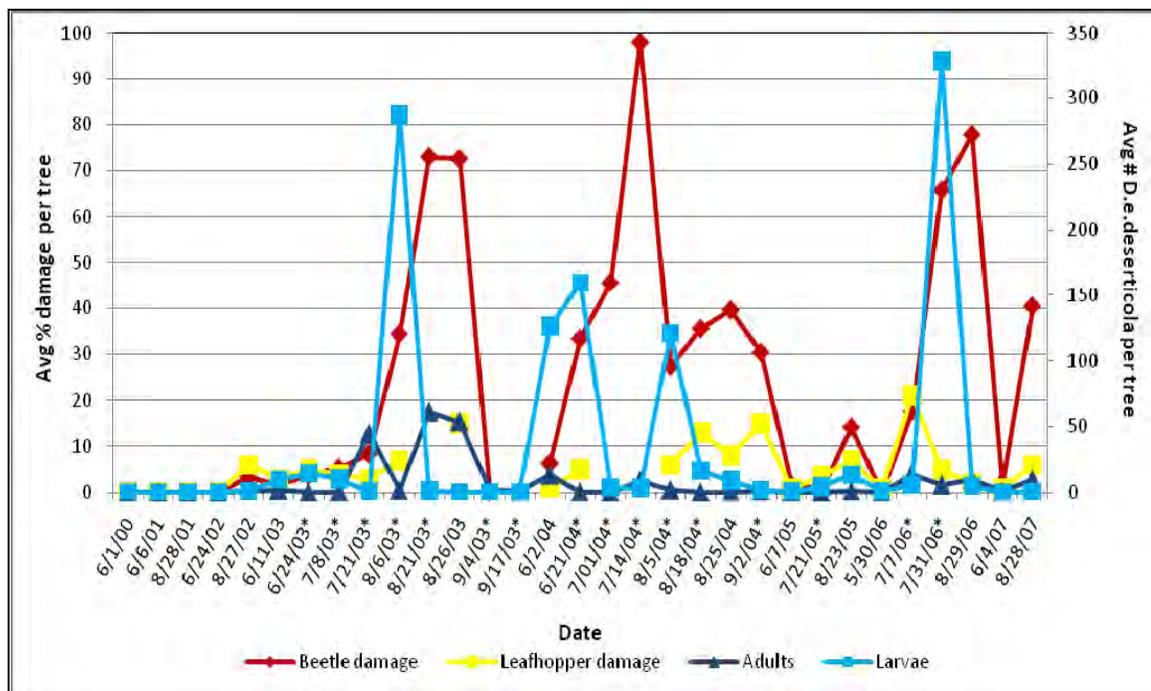


Figure 7.—Average percentage of foliar damage caused by the biocontrol beetle and the leafhopper in August and the average number of *D.e. deserticola* adults and larvae per tree in June and August from 2000 through 2007; Pueblo, CO.

values). In fact, these two variables showed no significant correlations with any other variables, however they also had very small sample sizes ($n=7$) due to the nature of the data, which was represented on an annual basis. No correlations were expected with precipitation since saltcedar is a phreatophyte, with deep roots that are in contact with groundwater, and therefore is less reliant on precipitation than other types of plants. The strongest correlations were between the percentage of damage and number of adults ($r=0.7427$), percent green foliage ($r= - 0.6669$), percent dead foliage ($r=0.8406$), and tree volume ($r=0.7427$). These results suggested that higher numbers of adult beetles influenced the amount of damage and that the percentage of damage to the saltcedar foliage in turn influenced the percentage of green and dead foliage. The positive correlation between damage and tree volume is not intuitive; as the damage increased, so did the cross area of the tree. This would seem to indicate that foliar damage by the beetle did not impact the growth of saltcedar.

TSC found that tissue damage and foliage color were often redundant data since foliage color (ie. dead or senescing) was typically used to determine the type of foliar damage (ie. beetle or leafhopper). Although foliage color may not have been the most accurate determinant for the cause of foliar damage, it was the best predictor that could be used in the field.

Wood without Foliage

The average percentage of wood without foliage per saltcedar tree as measured in August of each year was relatively constant, ranging from 13 to 19 percent, until 2004 when it increased substantially to 55 percent. The percentage of wood without foliage decreased to 38 percent in 2005 and remained around this level until the end of the study. The drastic increase in the amount of wood without foliage followed or coincided with an initial peak in the average number of adult and larvae beetles in 2003 and 2004 as shown in Figure 8 and supported by significant, though relatively weak, correlations (adult $r=0.1550$, $P=0.007$; larvae $r=0.2859$, $P=0.000$).

The percentage of wood without foliage increased statistically from 2000 to 2007 (see Table 2 for statistical results and P-values). The mean difference and standard deviation between paired samples used in the statistical comparisons for wood without foliage and other variables are shown in Table 3. There was also a significant increase from 2002 to 2003 and from 2003 to 2004. From 2004 to 2005, the percentage of wood without foliage decreased significantly, but not to levels as low as before 2004.

Significant correlations were found between the percentage of wood without foliage and all variables except senesced foliage, reproductive status, tree volume, plant canopy, and precipitation. None of the correlations were particularly strong.

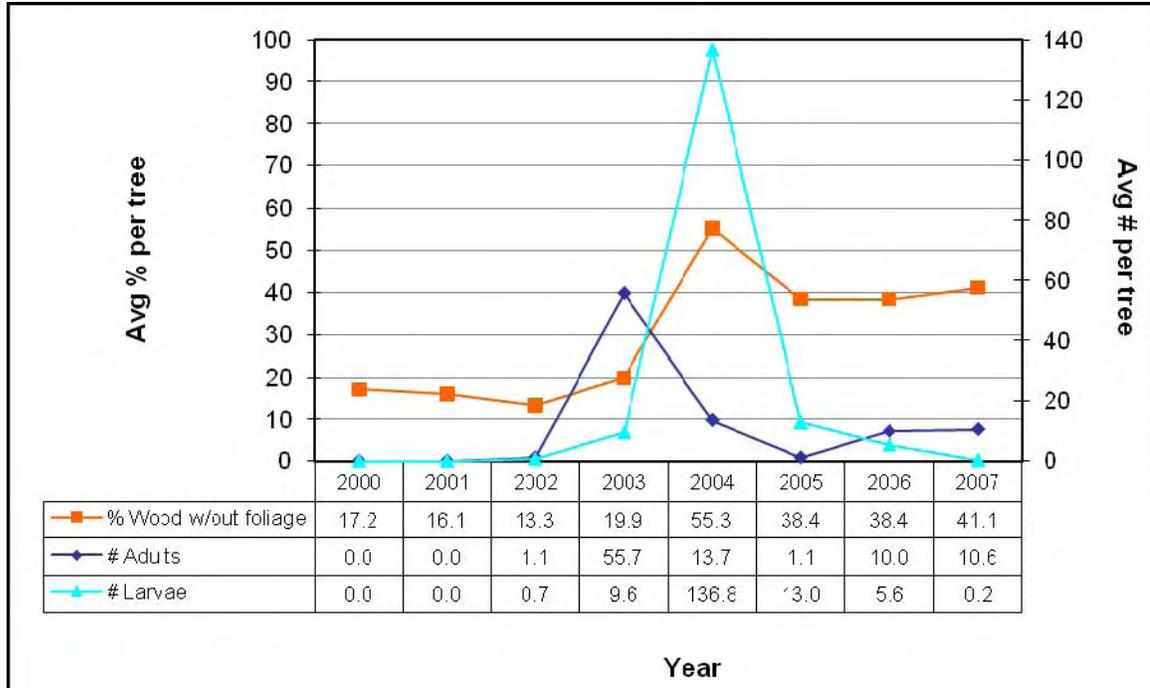


Figure 8.—Average percentage of wood without foliage per tree in August and average number of *D.e. deserticola* adults and larvae per tree per year from 2000 through 2007; Pueblo, CO.

Table 2.—Statistical results for selected variables comparing consecutive years and Year 1 (2000) to Year 8 (2007) of the study; Pueblo, CO. Alpha = 0.05.

Years	Wood w/out Foliage	Green foliage	Dead foliage	Reproductive Status ¹	Tree Volume
2000 vs 2001	00=01 P=0.650 ³	00>01 P<0.001 ²	00=01 P=0.054 ³	00=01 0.75>P>0.5	00<01 P<0.001 ²
2001 vs 2002	01=02 P=0.272 ³	01=02 P=0.442 ²	01<02 P<0.001 ³	01=02 0.5>P>0.25	01<02 P<0.001 ²
2002 vs 2003	02<03 P=0.019 ²	02>03 P<0.001 ³	02<03 P<0.001 ²	02=03 0.25>P>0.10	02=03 P=0.791 ³
2003 vs 2004	03<04 P<0.001 ²	03<04 P=0.017 ²	03>04 P<0.001 ²	03>04 P<0.001	03>04 P<0.001 ²
2004 vs 2005	04>05 P<0.001 ²	04<05 P=0.0 ²	04>05 P<0.001 ²	04<05 P<0.001	04<05 P<0.001 ²
2005 vs 2006	05=06 P=0.920 ²	05>06 P<0.001 ³	05<06 P<0.001 ³	05>06 P<0.001	05>06 P<0.001 ²
2006 vs 2007	06=07 P=0.551 ²	06<07 P<0.001 ²	06>07 P<0.001 ²	06<07 P<0.001	06<07 P<0.001 ²
2000 vs 2007	00<07 P<0.001 ²	00>07 P=0.0 ²	00<07 P=0.0 ²	00=07 0.9>P>0.75	00<07 P<0.001 ²

¹McNemar's test; ²Paired t-test; ³Signed rank test

Highlighted boxes = significant difference at the 95% confidence level

Table 3.—Mean difference and standard deviation between paired samples of selected variables comparing consecutive years and Year 1 (2000) to Year 8 (2007) of the study; Pueblo, CO.

Years	Wood w/out foliage (%)		Green foliage (%)		Dead foliage (%)		Tree volume (m ²)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
00vs01	2.17	21.40	39.79	26.50	-3.47	13.36	-2.47	5.83
01vs02	2.59	12.79	-3.79	28.42	-21.15	20.06	-3.95	8.22
02vs03	-7.39	18.67	39.89	35.22	-50.42	35.88	-0.56	4.79
03vs04	-35.64	27.30	-11.69	29.20	36.92	30.81	4.62	5.13
04vs05	16.93	18.85	-45.41	27.29	28.94	25.29	-9.26	11.84
05vs06	0.32	19.15	61.35	35.11	-68.71	35.92	10.47	9.60
06vs07	-2.68	27.48	-35.26	27.48	40.38	29.36	-8.56	8.23
00vs07	-24.75	30.75	47.92	23.84	-39.08	23.55	-10.06	11.26

The statistical increase in wood without foliage over time and the drastic increase with peak numbers of beetles appeared to indicate a long-term effect on the health of saltcedar from the biocontrol.

The percentage of wood without foliage appeared to be one of the best parameter for hypothesizing long-term effects of the beetles since many other variables were more point-in-time measurements. In a few cases there was a reduction in the amount of wood without foliage from year to year, which would indicate that wood that had no foliage was not necessarily dead wood.

Foliage Color

The average percentage of green and dead foliage per tree, as measured during the August data set, was variable from year to year. As would be expected, green and dead foliage showed an inverse relationship ($r = -0.7742$, $P = 0.000$) as demonstrated in Figure 9. Senescing foliage was highest early in the study in 2001 and 2002. The percentage of wood without foliage was not strongly correlated with foliage color (Figure 9), with correlation coefficients of -0.2489 ($P = 0.000$) for green foliage and 0.1841 ($P = 0.001$) for dead foliage.

Based on Figure 10, foliar color appeared to be linked with the estimated number of beetles, with dead foliage highest and green foliage lowest when adult and/or larval beetle populations were highest. Correlations were stronger between adult populations and green ($r = -0.5061$) and dead ($r = 0.6750$) foliage than with larval populations ($r = -0.2656$ and 0.3655 , respectively), however.

Statistically, there was a significant difference in the percentage of green foliage between all consecutive years except 2001 and 2002 (Table 1). Green foliage either increased or decreased from year to year, which shows the variability in the data for this parameter. This phenomenon was similar for the percentage of dead foliage, which also significantly increased or decreased from year to year, except between 2000 and 2001 (Table 2). Overall, the percentage of foliage that was green significantly decreased from Year 1 to Year 8 and the percentage of foliage that was dead significantly increased from Year 1 to Year 8. See Table 3 for mean

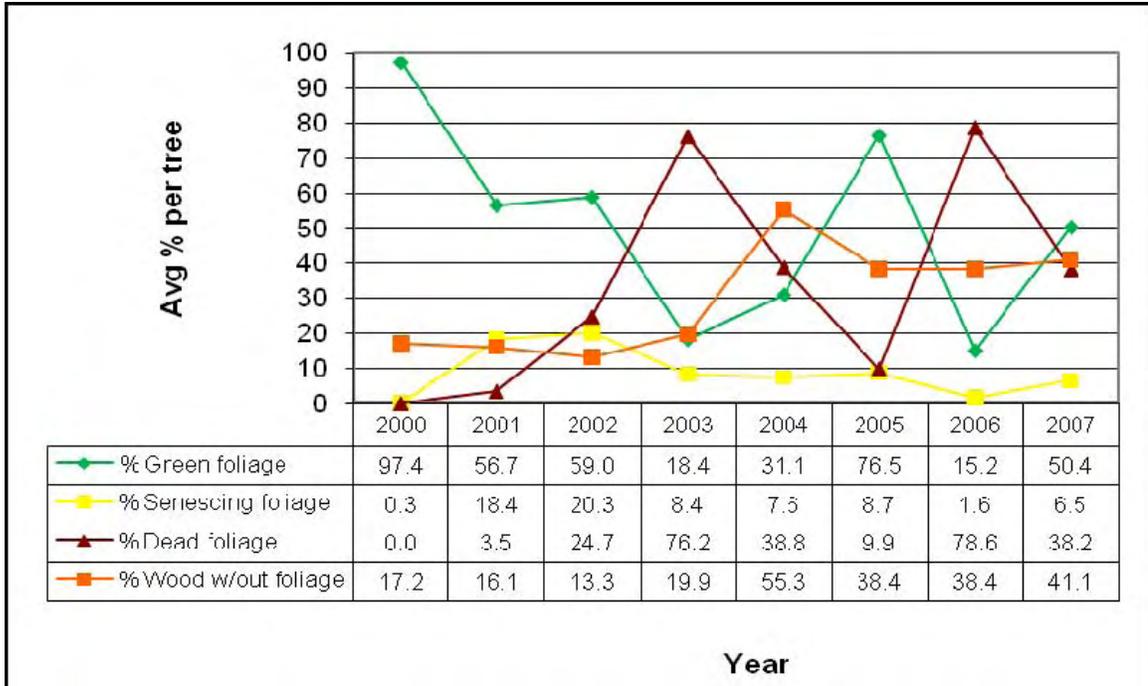


Figure 9.—Average percentage of foliage that was green, senescing, and dead and the average percentage of wood without foliage per tree in August from 2000 through 2007; Pueblo, CO

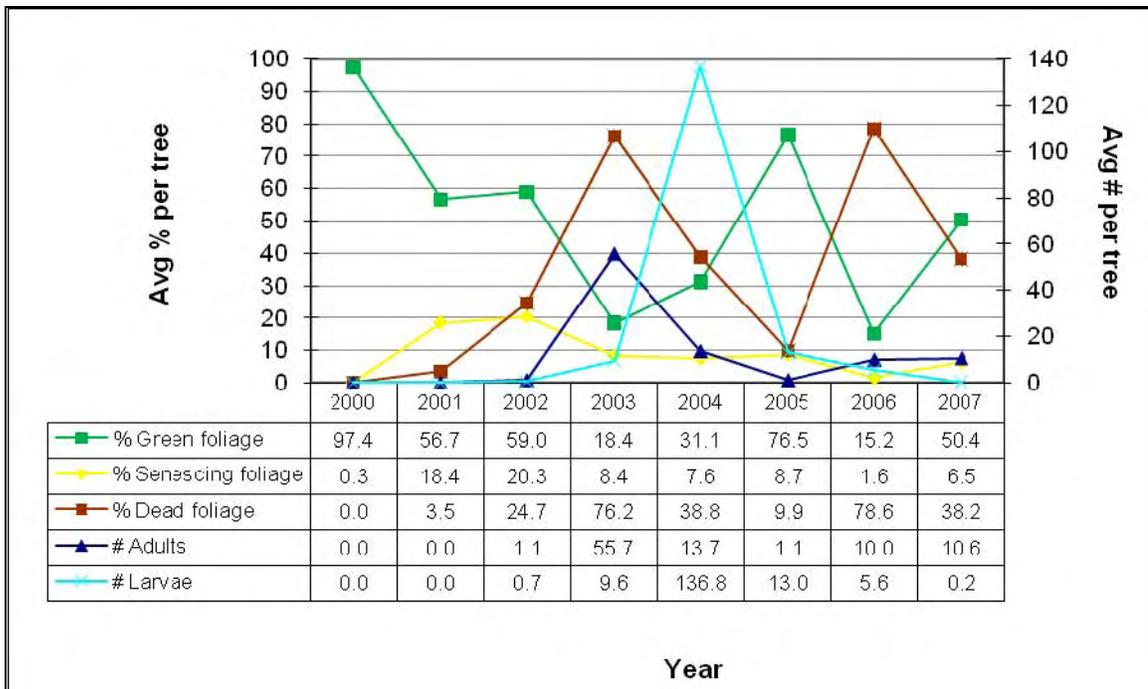


Figure 10.—Average percentage of foliage that was green, senescing, and dead and average number of *D.e.deserticola* adults and larvae per tree per year from 2000 through 2007; Pueblo, CO

difference and standard deviation between paired samples used in the statistical comparisons for green and dead foliage. These statistical results suggested that beetles may have impacted foliage over time.

Significant correlations were found for the percentage of green foliage with all variables except percentage of senesced foliage, reproductive status, percentage of native grass understory, and precipitation. Results were similar for the percentage of dead foliage, except that tree volume also showed no correlation. Not surprisingly, the percentage of damage by the beetle was strongly correlated with both. The percentage of senescing foliage was only found to be significantly correlated with the percentage of damage, tree volume, and plant canopy.

Regrowth

The percentage of foliage that had regrown following herbivory by the beetle was low in the early years of the study before the insect population began to thrive. Regrowth did not show an obvious correlation with the beetle population (Figure 11), however regrowth was most apparent in years that the larval populations were highest, and showed the strongest correlation with number of larvae ($r=0.5902$, $P=0.000$) than with any other variable. Consumption of foliage during the larval stage of the beetle's life cycle causes the most damage due to girdling of branches, so more regrowth would be expected when herbivory damage was presumably highest.

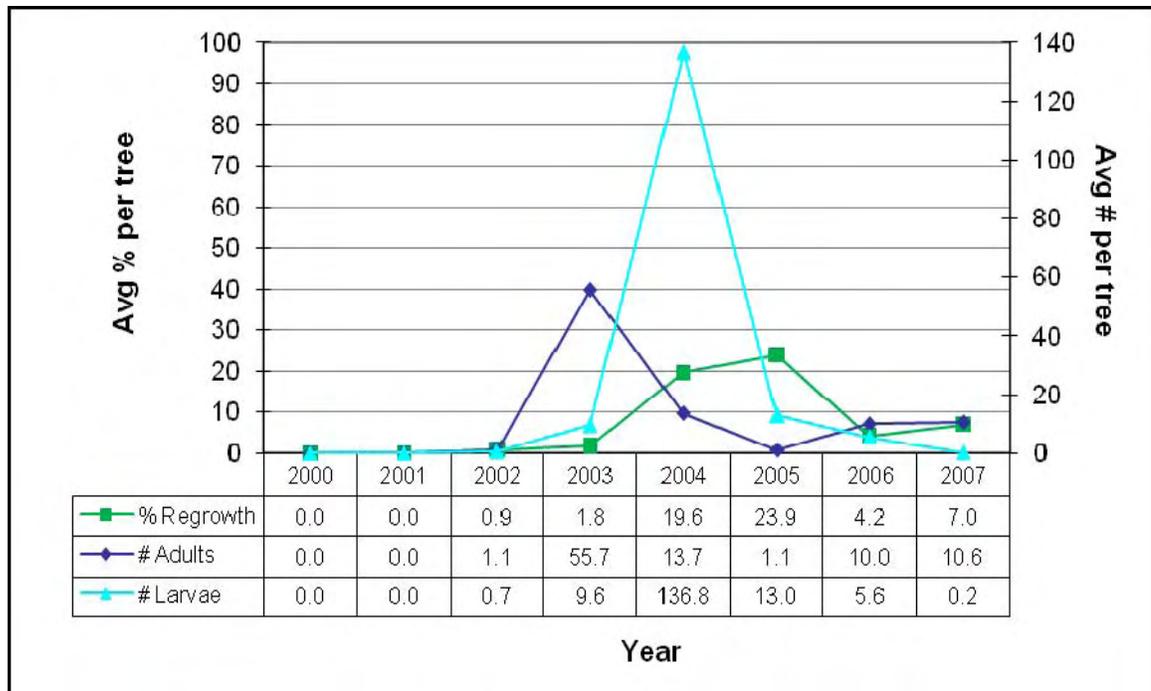


Figure 11.—Average percentage of regrowth following herbivory by the biocontrol in August and the average number of *D.e.deserticola* adults and larvae per year from 2000 through 2007; Pueblo, CO.

Significant correlations were found between the percentage of regrowth and all variables except the percentage of senescing foliage, reproductive status, and precipitation. Regrowth did show a positive correlation with year ($r=0.5324$), increasing over time from 0.0 to 7.0 percent, with a high of 23.9 percent in 2005.

Reproductive status

The percentage of trees that were reproducing, which was an average of the data collected in both June and August of each year, was highly variable from year to year, ranging from a minimum of 17.1 percent in 2004 to a maximum of 90.2 percent in 2000. Reproductive status did not appear to be related to the health of the tree as represented by the average percentage of wood without foliage (Figure 12). There did appear to be an inverse relationship to the estimated number of larvae for a few years (Figure 13), and some correlation – though not statistically significant – was found ($r= -0.6654$, $P=0.103$), which could have been attributed to a short-term impact by the beetle on flowering. In general, though, results did not indicate a long-term effect from the biocontrol on the reproductive capability of saltcedar.

Statistical comparisons between years showed no significant difference in the percentage of flowering trees between Year 1 (2000) and Year 8 (2007) of the study (Table 2). However, there were significant differences - both increases and decreases - in the percentage of flowering trees between consecutive years from 2003 to 2007. These results indicated variability in the reproductive status of saltcedar during the study period, but no overall change from beginning to end. No significant correlations were found with any of the other variables.

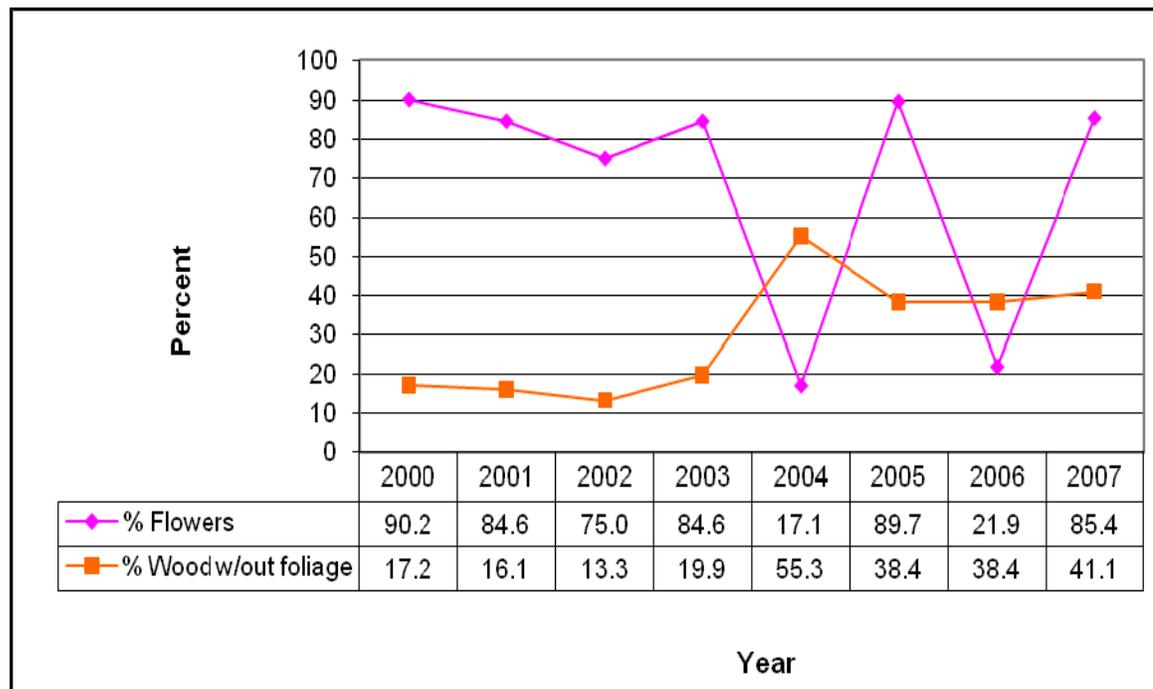


Figure 12.—The percentage of trees with flowers, buds, or seeds and the average percentage of wood without foliage per tree from 2000 through 2007; Pueblo, CO.

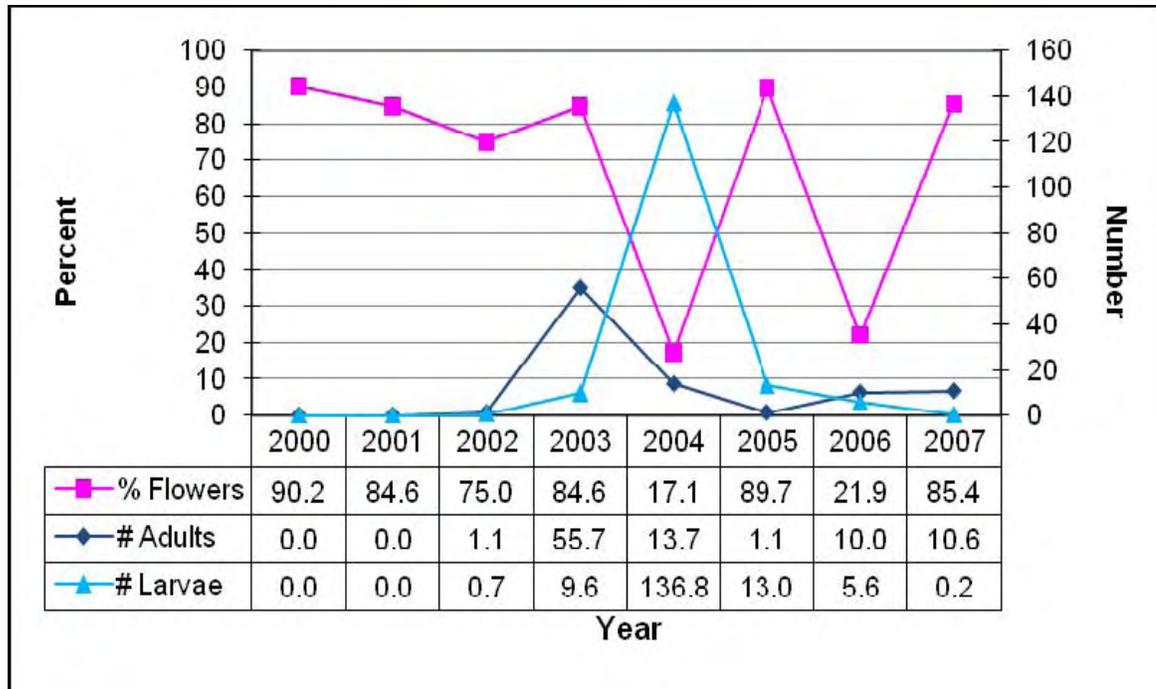


Figure 13.—The percentage of trees with flowers, buds, or seeds, and the average number of *D. e. deserticola* adults and larvae per year from 2000 through 2007; Pueblo, CO.

Tree volume

The average tree volume, as measured by the cross area (height x diameter) of saltcedar trees in August of each year, was somewhat variable over the study period. As mentioned, the methodology for measuring height was revised in 2005 in an attempt to limit variability, although the new method didn't lead to a noticeable difference. Tree volume measured only live plant material and the variability in cross area may have been a true reflection of the changing condition of foliage over the study period. There may have been some short-term damage from larvae, particularly in 2004, as shown in Figure 14, though correlation coefficients between tree volume and the number of adult ($r=0.1514$, $P=0.008$) and larval ($r=0.0906$, $P=0.112$) beetles showed extremely weak relationships over the course of the study. Based on graphical representation, there appeared to be a weak correlation between cross area and percent wood without foliage (Figure 15), with tree volume higher when the amount of wood without foliage was lower (i.e. live foliage was higher). However, statistical analysis found no correlation between the two variables ($r=0.0062$, $P=0.909$).

There was a significant increase in tree volume between 2000 and 2007 (Table 2). The mean difference and standard deviation between paired samples used in the statistical comparisons for tree volume are shown in Table 3. The average cross area varied significantly, both increasing and decreasing, between all consecutive years except 2002 and 2003. Significant correlations were found between tree volume and number of adult beetles, percent damage, percent green and senesced

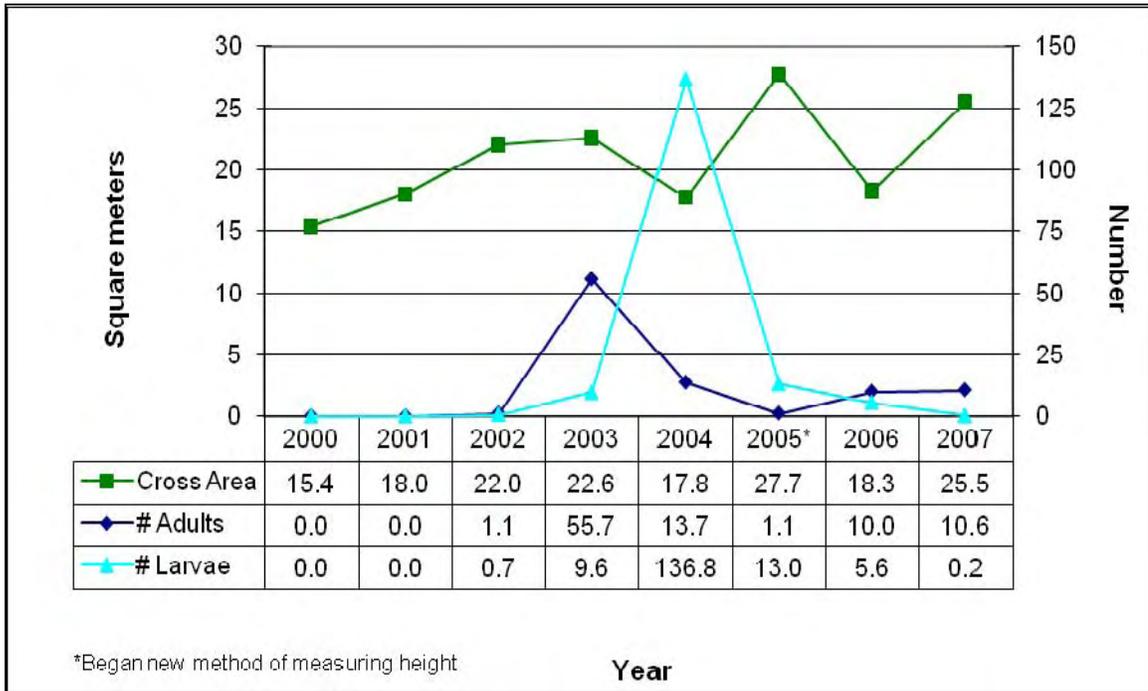


Figure 14.—Average cross area (height x diameter) of saltcedar and the average number of *D.e.deserticola* adults and larvae per year from 2000 through 2007; Pueblo, CO.

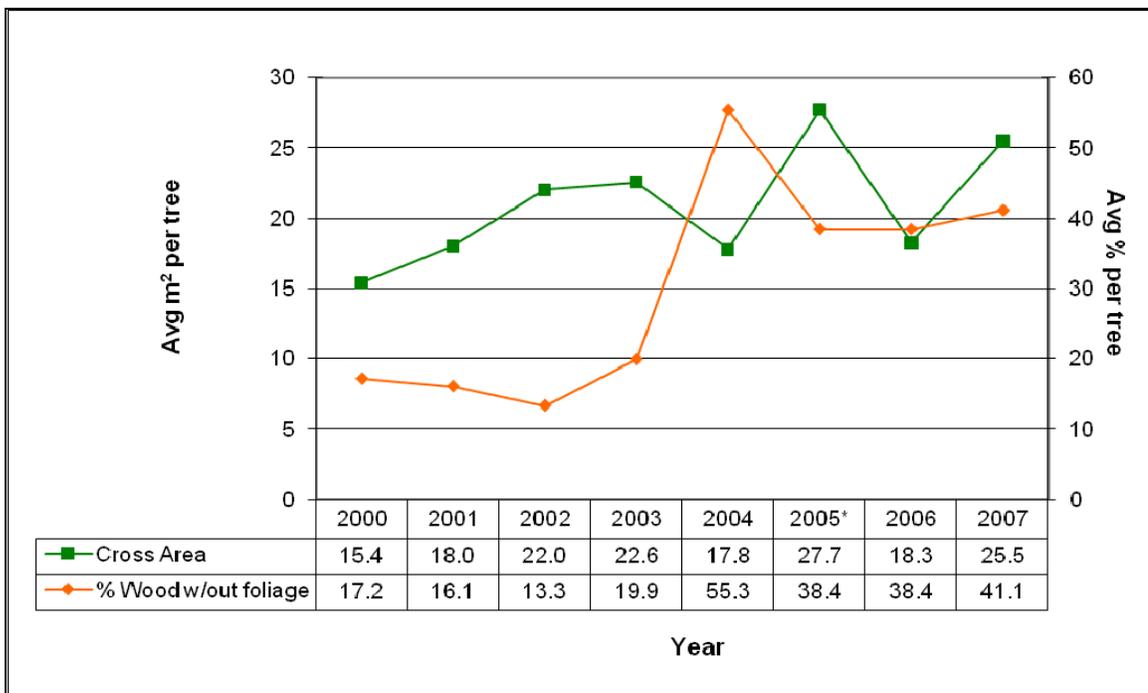


Figure 15.—Average cross area (height x diameter) of saltcedar and the average percentage of wood without foliage per tree from 2000 through 2007; Pueblo, CO.

foliage, percent regrowth, and plant canopy. Percent damage by the beetle showed the highest correlation with tree volume ($r=0.7427$). Although tree volumes varied from year to year, and although foliar damage appeared to affect tree volume, there was an overall increase in cross area from the beginning of the study to the end. This would suggest that the beetles did not completely limit the growth of some saltcedar within the study site.

Plant canopy

The leaf area index (LAI) was collected from 2003 to 2006 to measure plant canopy using the light bar. Essentially, a higher LAI related to a higher canopy cover. The average LAI increased from 2003 to 2005, then decreased in 2006 (Figure 16). There was no correlation observed between plant canopy and the percentage of wood without foliage. Based on the graph in Figure 17, there appeared to be an inverse correlation with the number of adult beetles, i.e. when LAI or plant canopy was highest, the number of adults was lowest. Although significant, there was a relatively weak relationship between these variables ($r= -0.2890$, $P=0.000$). Interestingly, there was a positive but relatively weak correlation between LAI and the number of larval beetles ($r=0.2614$, $P=0.001$), which indicated that plant canopy was highest when larval populations were highest.

The differences in LAI values were statistically significant between all consecutive years as well as between the first year of collecting this type of data and the last (Table 4). Even though values in 2006 decreased from 2005, there was an overall increase in LAI over time, which would indicate no effect from the

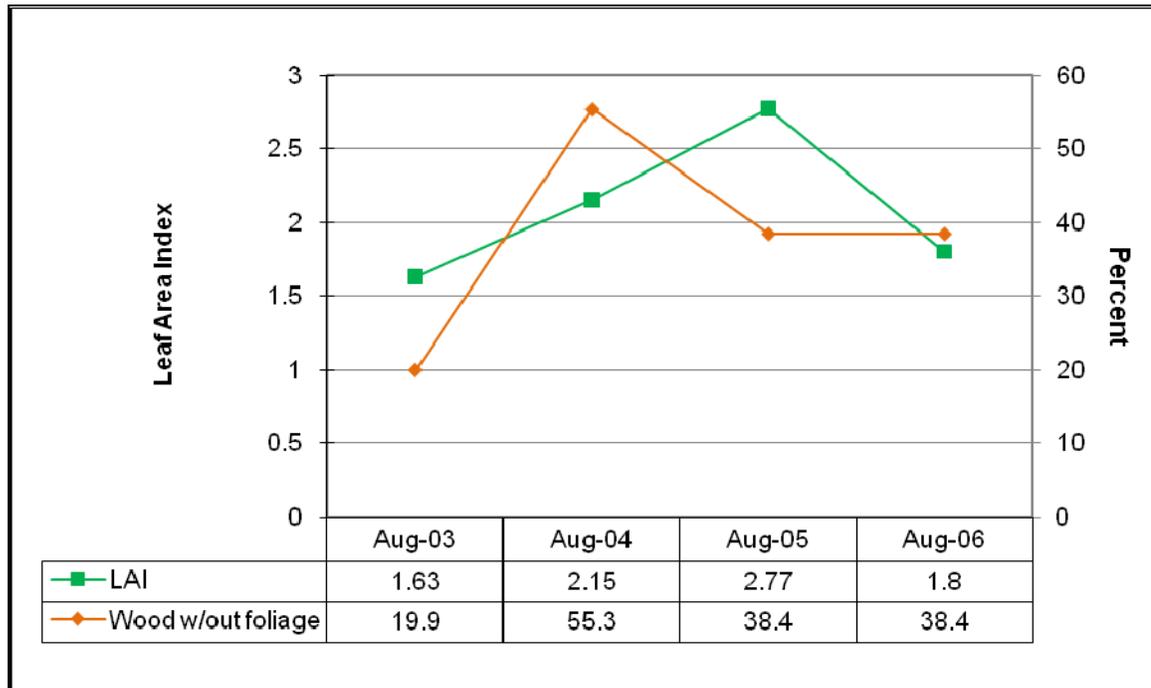


Figure 16.—Average leaf area index and the average percentage of wood without foliage per tree in August 2003 to 2006; Pueblo, CO.

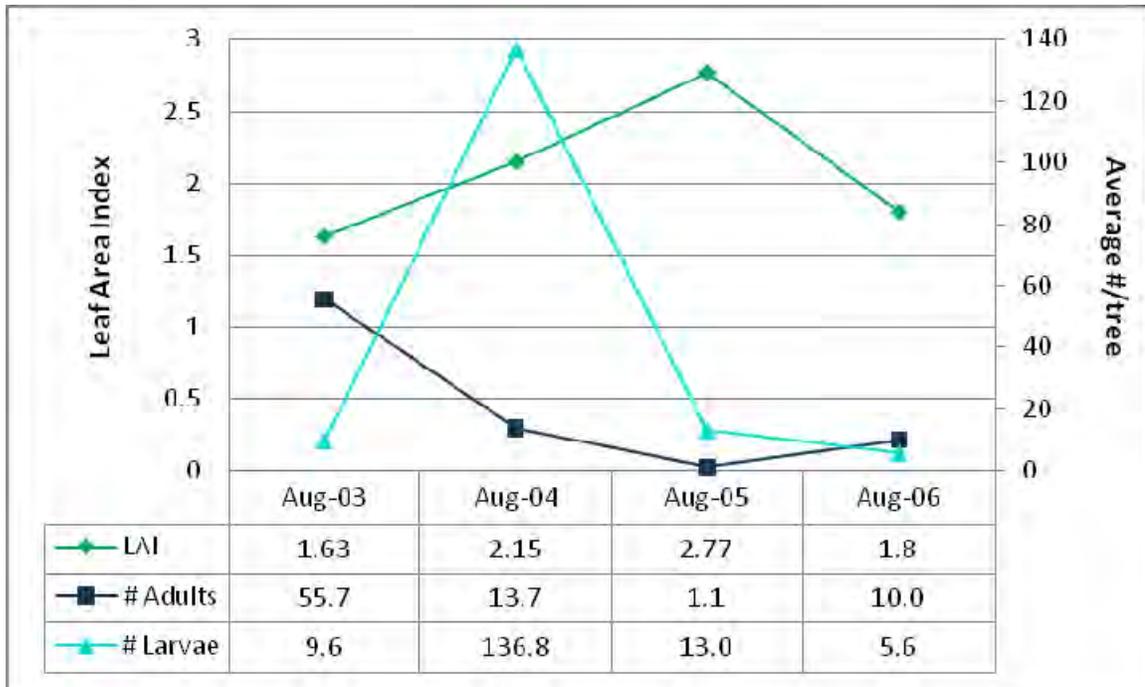


Figure 17.—Average leaf area index per saltcedar in August and the average number of *D. e. deserticola* adults and larvae per tree per year from 2003 to 2006; Pueblo, CO.

Table 4. Statistical comparisons (alpha = 0.05) and mean difference and standard deviation between paired samples for plant canopy comparing consecutive years and Year 1 (2003) to Year 3 (2006) of the light bar study; Pueblo, CO.

Plant Canopy Leaf Area Index (LAI)			
Years	P	Mean	SD
2003vs2004	03<04 P<0.001 ¹	-0.527	0.72
2004vs2005	04<05 P<0.001 ²	-0.618	0.91
2005vs2006	05>06 P<0.001 ²	0.966	1.05
2003vs2006	03<06 P=0.042 ¹	-0.195	0.59

¹Paired t-test; ²Signed rank test

Highlighted boxes = significant difference at the 95% confidence level

beetle on the canopy cover of saltcedar. Significant correlations were found between plant canopy and all variables except for percent of wood without foliage, reproductive status, percent of introduced forb understory, and precipitation, though none of the correlations were particularly strong.

Results weren't necessarily consistent with those found through measurements of wood without foliage, which significantly increased over the study. An increase in wood without foliage conversely relates to a decrease in the amount of wood with foliage, which would presumably translate to a decrease in plant canopy.

The accuracy of light bar readings was in question due to the specific conditions that were required to obtain measurements. Readings could only be collected from 10 am to 2 pm while the sun was highest and only if skies were clear. The sky conditions were required to be consistent from the beginning of the reading to the end for each tree, therefore the presence of clouds often created problems with collecting consistent readings. These specifications restricted the ability to obtain reliable data due to limited staff and field days.

Associated woody vegetation

Nearest neighbor data (i.e. nearest three woody species > 2.5 cm diameter) were collected in 2000, 2003, 2004, and 2005. In 2005, data were collected inconsistently as compared to other years and were therefore not included in comparisons. There was no perceptible change in data from 2000 to 2004. The most common species by far in 2000, 2003, and 2004 was saltcedar (counts were 113, 112, and 110, respectively). The next most common species was Russian olive (3, 7, and 7), followed by greasewood, willow, and juniper. In 2008, the woody invasive species (i.e. saltcedar and Russian olive) were removed from the original project site. Following this removal effort, rabbit brush was the most common woody species (though generally less than 2.5 cm diameter) remaining, drastically changing the composition from a monotypic saltcedar stand.

Photos

Photos comparing the 100 trees in 2001 and 2007 are shown in Appendix D. In a few cases, 2001 photos were not available and photos from other early years were used.

The photographs provided an excellent example of the degradation of saltcedar in the study area over the monitoring period. Foliar damage from the beetle was evident in the comparisons of the majority of photos between the beginning and the end of the study. Figure 18 shows a few cases of noticeable declines in the health of individual saltcedar from 2001 to 2007.

Associated Understory

Total cover

The total percent cover of plant, litter, and bare soil as estimated in August were variable from year to year (Figure 19). There was a statistically significant change in plant cover between all consecutive years (Table 5). The mean difference and standard deviation between paired samples used in the statistical comparisons for associated understory total and relative cover are shown in Table 6. Total plant



2001



2007



2001



2007



2001



2007

Figure 18.—Photos showing comparisons in the health of saltcedar samples from 2001 to 2007; Pueblo, CO.

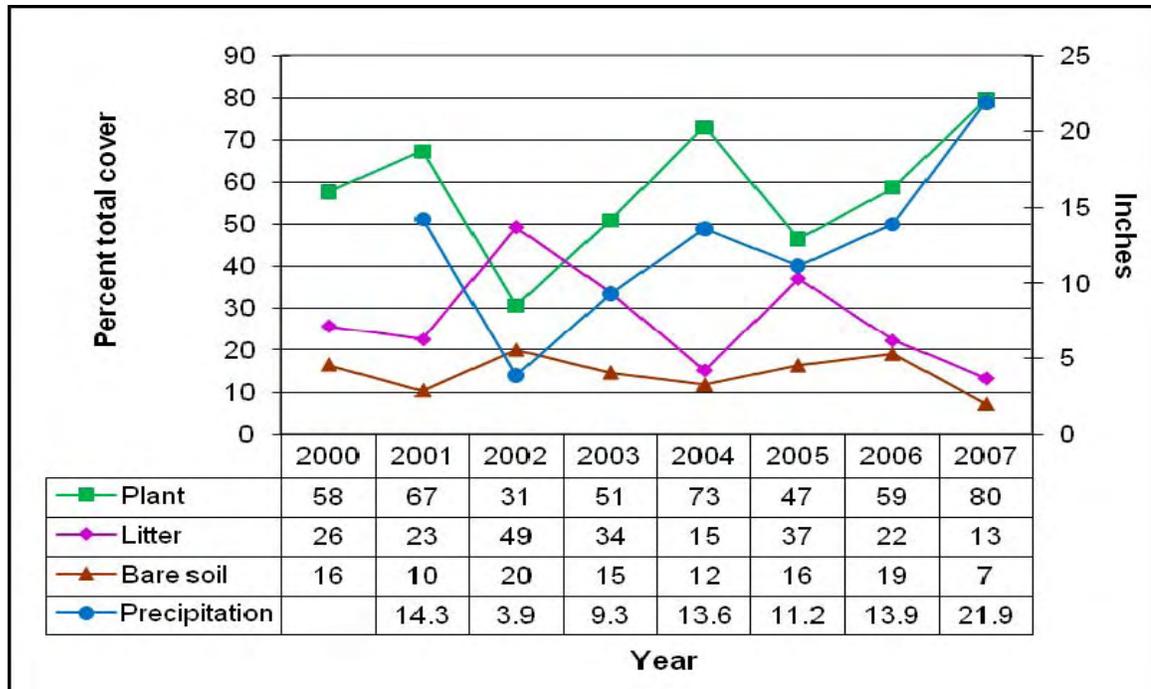


Figure 19.—The average percent total cover of plant, litter, and bare soil in quadrat measurements of understory associated with saltcedar samples in August of 2000 to 2007 and cumulative annual precipitation from 2001 through 2007; Pueblo, CO.

Table 5.— Statistical results for associated understory total and relative cover comparing consecutive years and Year 1 (2000) to Year 8 (2007) of the study; Pueblo, CO. Alpha = 0.05.

Years	Plant total cover (%)	Litter total cover (%)	Bare total cover (%)	Native grass relative cover (%)	Introduced forb relative cover (%)	Native plant relative cover (%)
2000 vs 2001	00<01 P=0.030 ²	00=01 P=1.0 ²	00>01 P=0.011 ¹	00=01 P=0.412 ²	00=01 P=0.484 ²	00=01 P=0.861 ²
2001 vs 2002	01>02 P=0.0 ¹	01<02 P<0.001 ¹	01<02 P=0.001 ²	01<02 P=0.027 ²	01>02 P=0.004 ²	01<02 P=0.002 ²
2002 vs 2003	02<03 P<0.001 ¹	02>03 P<0.001 ¹	02>03 P=0.026 ¹	02>03 P<0.001 ²	02<03 P<0.001 ²	02>03 P<0.001 ²
2003 vs 2004	03<04 P<0.001 ¹	03>04 P<0.001 ²	03=04 P=0.096 ²	03>04 P<0.001 ²	03<04 P<0.001 ²	03>04 P<0.001 ²
2004 vs 2005	04>05 P<0.001 ¹	04<05 P<0.001 ¹	04<05 P=0.048 ²	04=05 P=0.387 ²	04=05 P=0.544 ²	04=05 P=0.789 ²
2005 vs 2006	05<06 P=0.007 ¹	05>06 P<0.001 ¹	05=06 P=0.130 ¹	05=06 P=0.561 ²	05=06 P=0.217 ²	05=06 P=0.289 ²
2006 vs 2007	06<07 P<0.001 ¹	06>07 P<0.001 ²	06>07 P<0.001 ¹	06=07 P=0.274 ²	06=07 P=0.053 ²	06=07 P=0.150 ²
2000 vs 2007	00<07 P<0.001 ¹	00>07 P=0.003 ¹	00>07 P=0.002 ¹	00>07 P=0.017 ¹	00<07 P<0.001 ²	00>07 P<0.001 ¹

¹Paired t-test; ²Signed rank test

Highlighted boxes = significant difference at the 95% confidence level

Table 6.—Mean difference and standard deviation between paired samples comparing total and relative cover of associated understory for consecutive years and Year 1 (2000) to Year 8 (2007) of the study; Pueblo, CO.

Years	Total plant cover (%)		Total litter cover (%)		Total bare cover (%)		Native grass cover (%)		Introduced forb cover (%)		Native plant cover (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
00vs01	-9.51	24.94	3.22	22.45	5.20	12.27	-3.73	22.66	-0.68	22.12	0.90	17.96
01vs02	36.48	22.70	-26.66	23.30	-9.08	16.73	-17.44	36.22	17.87	33.98	-19.54	35.66
02vs03	-20.35	22.87	16.23	22.28	4.13	11.30	26.63	32.36	-25.21	31.63	23.98	31.28
03vs04	-22.10	25.97	18.28	23.47	3.83	15.03	14.45	21.03	-16.25	19.87	17.53	20.54
04vs05	26.10	38.51	-21.64	34.34	-4.46	13.86	-3.21	21.70	3.32	25.17	-2.18	20.82
05vs06	-12.31	26.76	15.08	23.96	-2.77	11.19	1.66	21.03	-4.60	24.66	2.25	20.12
06vs07	-20.80	16.37	8.95	18.05	11.85	12.31	-4.05	21.00	6.65	21.10	-5.41	21.61
00vs07	-21.76	26.68	12.46	25.63	9.29	17.74	14.04	36.04	-19.29	31.97	18.32	31.27

cover increased significantly from 58 percent in Year 1 of the study to 80 percent in Year 8. This increase in understory plant cover could indicate an effect from the beetle, with associated vegetation presumably increasing as the amount of light reaching the understory increased with less canopy cover, as indicated by the increase in wood without foliage. However it is difficult to determine if this increasing trend would have continued if the study were carried out over a longer period of time since there was so much variation in the percentage of understory plant cover, which either increased or decreased from one year to the next. It was unclear if the change that occurred from the beginning of the study to the end was attributed to foliar damage by the beetle. Total plant cover was more likely linked to precipitation, as shown in Figure 19 and supported by a strong statistical correlation ($r= 0.9226$, $P=0.003$).

The percentage of total litter cover was significantly different between all consecutive years except 2000 and 2001 (Table 5) and appeared to have an inverse relationship to plant cover (Figure 19). There was an overall decrease in litter cover from 26 percent in 2000 to 13 percent in 2007.

The values for total cover of bare soil were somewhat more consistent relative to the other two variables, yet significant differences were found between consecutive years with the exceptions between 2003 and 2004 and between 2005 and 2006. There was a significant decrease in bare soil cover from Year 1 to Year 8 of the study.

Relative Cover

Native species initially dominated the herbaceous understory as compared to introduced species based on relative percent cover (Figure 20). In 2004, this scenario shifted, and introduced species dominated the associated understory plots throughout the remaining period of study. There were statistically significant differences in native plant cover between consecutive years from 2001 to 2004 (Table 5). There was also a significant decrease in native plant cover from the beginning of the study to the end, which would conversely relate to a significant

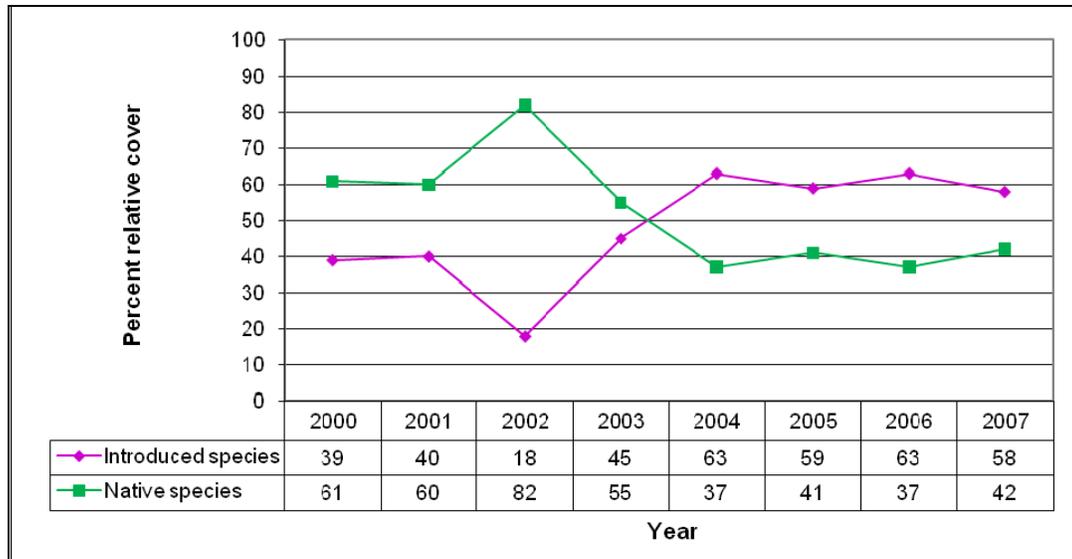


Figure 20.—Percent relative cover of native and introduced herbaceous species in the understory associated with saltcedar samples in August from 2000 to 2007; Pueblo, CO.

increase in the relative cover of introduced species. The most common native species based on relative percent cover throughout the study period were alkali sacaton (*Sporobolus airoides*), scratch grass (*Muhlenbergia asperifolia*), and salt grass (*Distichlis spicata*). The most common introduced species based on percent relative cover were kochia (*Kochia scoparia*), whitetop (*Cardaria draba*), and lambsquarters (*Chenopodium* spp.).

Relative to other lifeforms, native grasses and introduced forbs were the predominant understory plants associated with the saltcedar sample trees (Figure 21). As such, results from statistical comparisons of the relative cover of native grasses between years were the same as results from native plant cover and results from comparisons of introduced forbs were exactly opposite. The percent relative cover of native grasses was significantly different between consecutive years from 2001 to 2004 and there was a significant decrease in native grass cover over the study period (Table 5). Conversely, the relative cover of introduced forbs was significantly different between consecutive years from 2001 to 2004 (with increases and decreases being the reverse of native grasses), and introduced forbs statistically increasing over time.

As demonstrated in Figure 22, native grasses and introduced forbs had a strong inverse relationship ($r = -0.8833$, $P = 0.000$). Both lifeforms appeared to be driven by precipitation, with relative cover of introduced forbs positively associated with annual precipitation, while native grasses showed a negative association. The large difference between native grass and introduced forb cover in 2002 was most likely attributed to the extremely low precipitation that year. Results indicated that introduced forbs were not as able to adapt to the drier conditions as were the

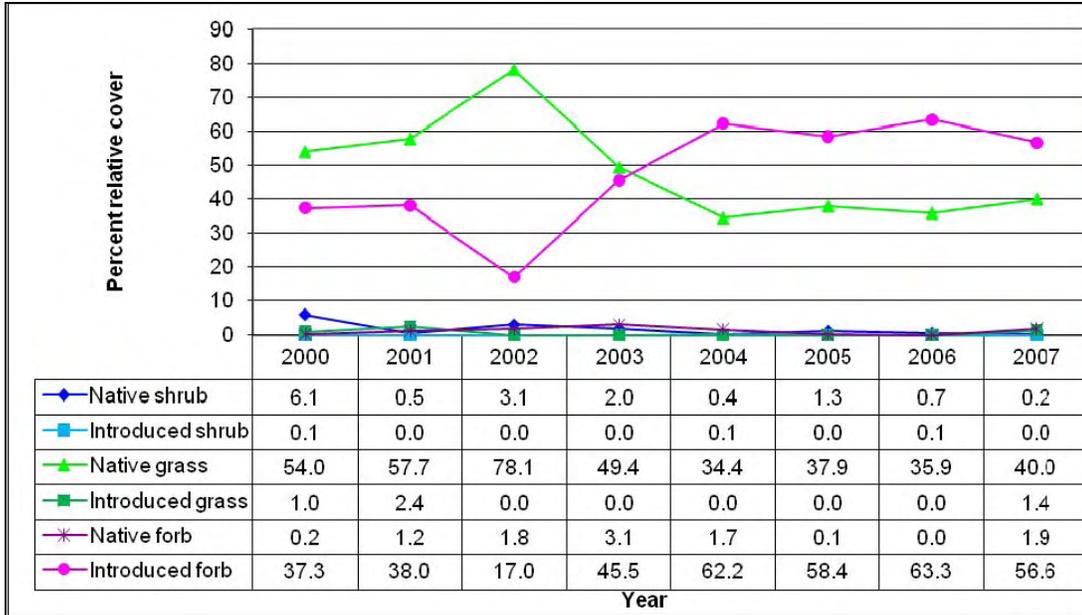


Figure 21.—Percent relative cover by lifeform of the herbaceous understory associated with saltcedar samples in August from 2000 to 2007; Pueblo, CO.

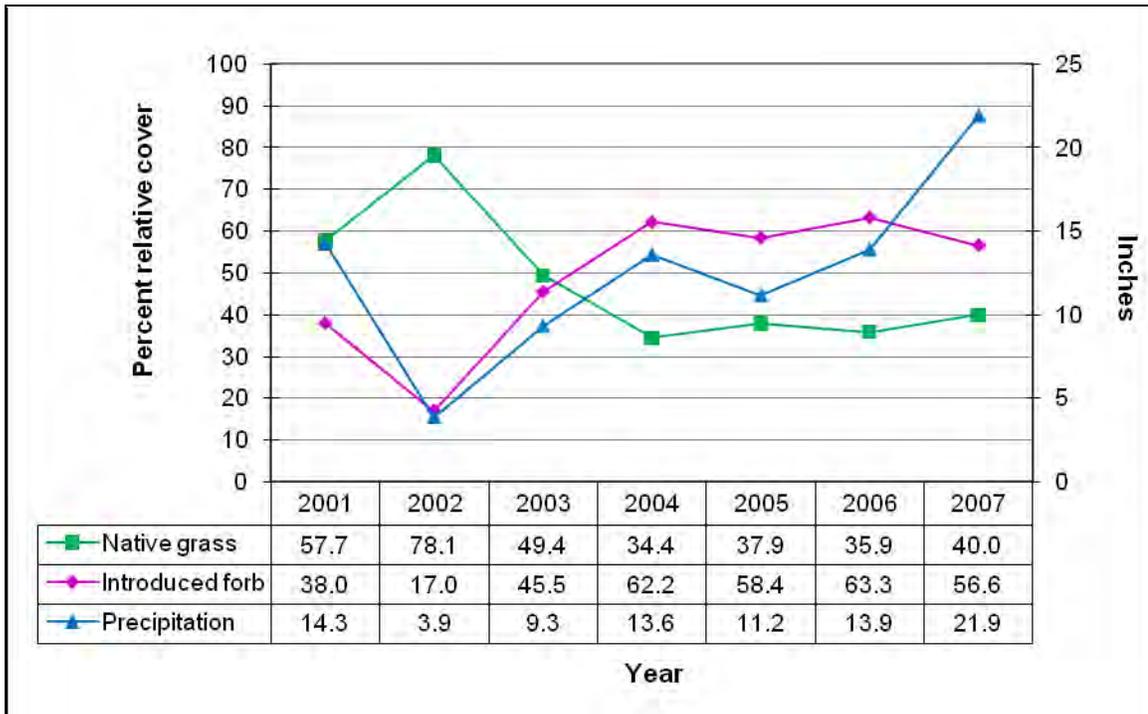


Figure 22.—Percent relative cover of native grasses and introduced forbs in the herbaceous understory associated with saltcedar samples in August and cumulative annual precipitation from 2001 to 2007; Pueblo, CO.

native grasses. Native grasses were also more likely to perform better with less competition from the introduced species. It should be noted, however, that plant cover in general was relatively low that year (Figure 19).

There also appeared to be somewhat of a positive correlation between relative cover of introduced forbs and wood without foliage (Figure 23), and statistical analysis indicated a relatively weak but significant relationship ($r=0.2428$, $P=0.000$). From 2005 on, all three variables – introduced forb cover, native grass cover, and wood without foliage – more or less level off. Significant inverse correlations were found between percent native grass cover and number of larvae, percent damage, percent wood without foliage, percent regrowth, plant canopy, percent introduced forb cover, and year. Although these correlations were all relatively weak, they did show that as variables indicating less foliage (with the exception of plant canopy) increased, native grasses decreased. Significant correlations were found between percent introduced forb cover and number of adults and larvae, percent damage, percent wood without foliage, percent plant canopy, percent green and dead foliage, percent regrowth, percent native grass cover, and year. Growth of introduced forbs may be more dependent on light reaching the understory based on correlation results, and especially as represented by wood without foliage, than native grasses. If this were the case, the amount of introduced forb cover would be expected to continue to dominate as the amount of wood without foliage increased due to beetle impact.

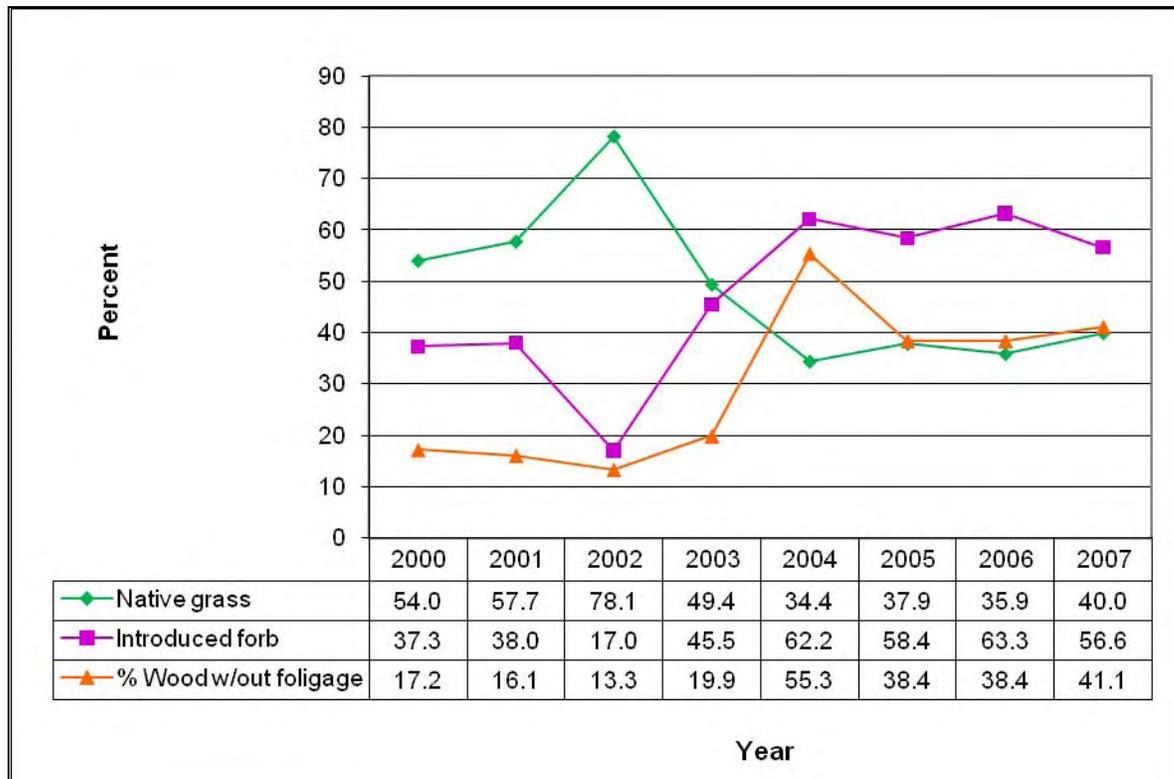


Figure 23.—Percent relative cover of native grasses and introduced forbs in the herbaceous understory and percentage of wood without foliage on associated saltcedar trees in August of 2000 to 2007; Pueblo, CO.

There appeared to be some evidence that the decrease in native species (i.e. native grasses) over the study period and related increase in introduced species (i.e. introduced forbs) were linked to changes in the foliar cover of associated saltcedar, and thus could be linked to an effect from the beetle. A number of other factors may have contributed to the results as well, including annual precipitation, drought, grazing herbivores, soil salinity, and resource management practices in the area.

Photos

Photos comparing quadrats in 2001 and 2007 are shown in Appendix E. Some of the 41 quadrat photos were not available; therefore only 38 photo comparisons are included. Figure 24 shows some photos that illustrate the shift from native species to introduced species (predominately kochia) in the understory over the study period.

Photo Stations

Photos comparing landscape views from 2004 or 2005 to 2007 are shown in Appendix F. In many cases, foliar damage to saltcedar stands was evident over time.

Conclusions

Original Tree Stand

The original 100 trees were revisited in 2007 and an abbreviated data collection was conducted. When comparing data from 2001 and 2007, average height significantly increased from 3.9 to 4.6 m and the percentage of wood without foliage significantly increased from 14.7 percent to 47.0 percent. These results indicated that the beetle did not affect the growth of the tree but did cause longterm damage to saltcedar foliage.

Forty-one trees were monitored for the duration of the study period and the data collected were analyzed for various parameters. A summary of the results from these analyses follow.

The adult and larval beetle populations (as calculated by averaging all available data sets annually) both peaked in 2003, 2004, and 2006. These years were also those that had the most comprehensive data sets, when additional data collection was conducted outside of the early June and late August visits. When using only

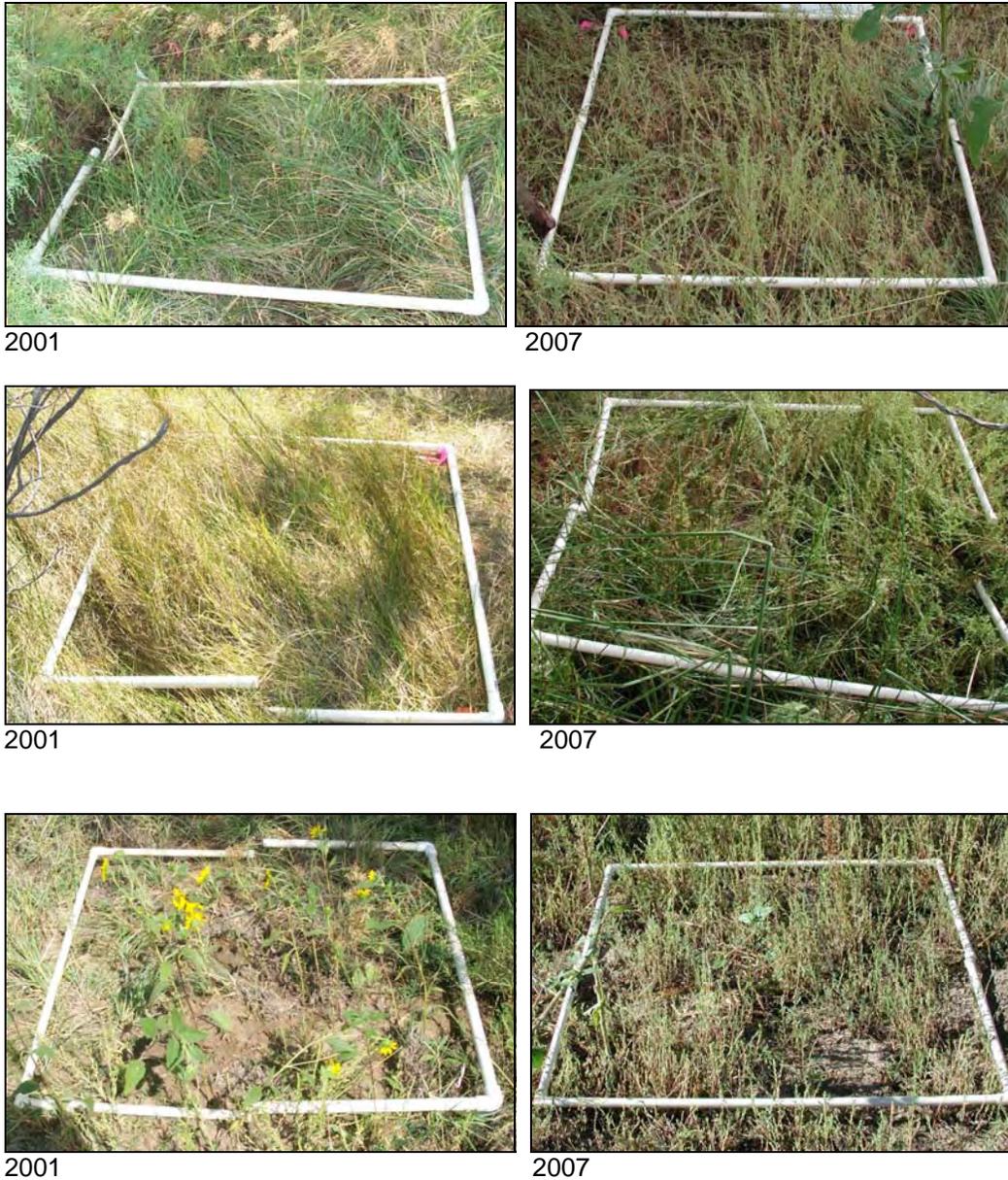


Figure 24.—Photo comparisons of quadrats showing the shift from native to introduced species from 2001 and 2007.

beetle population data that was collected in association with this study, peak years for both larvae and adults were 2003 and 2004. There was an increasing trend in beetle numbers after release in 2001 followed by a decreasing trend from 2004 through 2007. The one-minute count method for estimating beetle populations provided relatively accurate numbers. A trial using this method was used to determine that the minimum value of whole tree range estimates (as opposed to the midpoint of each range) was the best to use in analysis. Statistical analysis examining possible effects of climate on beetle numbers indicated that total larval populations were higher following warmer winters (ie. higher degree days

October-March). No other climate factors significantly affected beetle populations. The branch data beetle counts provided evidence and numbers of eggs but was not important for providing data to supplement estimates from the whole tree. Annually, the peak tissue damage to saltcedar generally followed peak larval populations by about 1 to 2 weeks.

The average percentage of wood without foliage per tree statistically increased over the study period from 17.2 percent in 2000 to 41.1 percent in 2007. The greatest increase in wood without foliage from one year to the next was 35.4 percent in 2004, which coincided with the highest estimates of larvae numbers. Significant correlations indicated the beetle had some effect on the percent of wood without foliage over the course of the study. Wood without foliage was probably the best parameter for assessing a long-term effect from the beetle on the health of saltcedar.

The average percentage of green foliage per tree significantly decreased from 97.4 percent in 2000 to 50.4 percent in 2007, while the average percentage of dead foliage significantly increased from 0 percent to 38.2 percent over the same period. Dead foliage was highest and green foliage lowest when the adult and/or larvae beetle populations were highest. These results also appear to support an impact from the beetle on saltcedar foliage. Regrowth, as defined by tissue that refoliated after herbivory by the beetle, was documented when larvae populations were up, and there was a moderately strong correlation with beetle numbers.

There was no statistical change in the reproductive status of saltcedar over the study period, although the percentage of trees with flowers was highly variable between years. No significant correlations were found between reproductive status and other variables. These results imply that the beetle did not affect reproductive capability of saltcedar in the long-term.

Tree volume significantly increased as represented by cross area (height x diameter) over the study period, from 15.4 m to 25.5 m. There were weak inverse relationships between cross area and the estimated number of larvae and adults (i.e. as the number of beetles increased, the cross area decreased). These results suggest a possible short-term affect by the beetle on tree volume, but not on the overall growth of saltcedar.

Plant canopy of saltcedar as measured by LAI statistically increased from 2003 to 2006, from 1.63 to 1.80, with a maximum LAI of 2.77 in 2005. These results suggested no impact on saltcedar foliage and were contrary to results from the percentage of wood without foliage, which showed that the amount of foliage on saltcedar significantly decreased. The light bar that was used to measure LAI required very specific conditions that were difficult to obtain for all readings, and therefore the accuracy of plant canopy data was questionable.

Saltcedar remained the most abundant woody species greater than 2.5 cm that was associated with sample trees from 2000 to 2004. Seemingly, a much longer study

period would be needed to detect a change in the composition of nearest neighbor species.

Visual observation of the site through photographs showed a perceptible decline in the health of saltcedar over the monitoring period.

Associated Understory

The total percentage of plant cover in the understory associated with saltcedar samples statistically increased from 58 percent in 2000 to 80 percent in 2007. Conversely, bare soil cover decreased from 16 to 7 percent, and litter cover decreased from 26 to 13 percent. The percentage of plant cover appeared to be related to the amount of cumulative annual precipitation.

The relative cover of native plant species decreased from 61 to 42 percent during the study period as the relative cover of introduced plant species increased from 39 to 58 percent. Introduced forbs and native grasses were the most common lifeforms found in the understory. From 2001 to 2006, graphs showed a positive association between the percentage of introduced forb cover and annual precipitation and a negative association between the percentage of native grass cover and precipitation, though correlations were not statistically significant at $\alpha=0.05$. These results suggest that native grasses were more adaptable to drier conditions. There were also statistically significant correlations between introduced forbs and variables that indicated decreasing foliage, such as number of adult and larvae beetles, percent damage, percent wood without foliage, and percent green and dead foliage. This may have meant that as beetles decreased the amount of foliage on the trees, the amount of light reaching the understory increased, which increased the cover of introduced forbs and simultaneously decreased the cover of native grasses. Although there appeared to be some evidence that decreasing cover of native grasses and increasing cover of introduced forbs were linked to effects from the beetles, other factors, such as climate factors and land management practices, may have contributed to the outcome as well.

The shift in the species composition of associated understory vegetation was visually documented in many of the photographs that were taken of quadrats used to collect data.

Photo Stations

Visual observation of the project site through photographs showed the apparent decline in the health of saltcedar on a landscape level over the monitoring period.

Summary

One problem TSC encountered was that data sets may not have captured a representative estimate of beetle population sizes in all years since the number of times that supplemental data was collected varied from year to year. During years that the number of visits to collect data was low, surges in populations at varying life stages may have been missed. Therefore, accurate correlations may not have been detected. A consistent, biweekly collection of biocontrol population estimates in order to provide a complete record is recommended for future studies of this nature.

A longer study would have been necessary to determine if the beetle would actually cause saltcedar mortality. Additional monitoring would also show if trends that were documented in the species composition of associated vegetation would continue and help to determine if these trends were a function of beetle impact. Over the eight year duration of this study, it appeared that the beetle did affect the health of saltcedar based on the condition of foliage, but did not completely limit the growth or reproductive ability of saltcedar. The beetle population, although variable in number from year to year, was sustained and appeared to be relatively stable at this location.

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**PEER REVIEW DOCUMENTATION
PROJECT AND DOCUMENT INFORMATION**

Document: Saltcedar Biocontrol at Pueblo, Colorado: Vegetation Monitoring Final Report

Date: June, 2010 Date Transmitted to Client: June, 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

Reviewers: Scott O'Meara, Fred Nibling

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: Fred L. Nibling Review Date: May 3, 2010
Signature
Reviewer: Scott O'Meara Review Date: May 5, 2010
Signature

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: Rebecca Siegle Date: June 14, 2010
Signature

Appendix A

Data forms for Original Tree Stand and Associated Understory

SC Biocontrol Consortium Vegetation Monitoring Data Form
Pueblo, Colorado

Tree #: _____

Data Form: Biomass/Herbivory

Date: _____

Observer: _____

Observer: _____

Recorder: _____

Time: _____

Tree Volume:

Height: ____ m
 Measure highest live point, in meters.

Diameter: N-S: ____ m E-W: ____ m
 Measure widest live point at cardinal directions, in meters.

Reproductive Status of Entire Tree:

Flowers? Y/N Include all stages (i.e. buds, open flowers, seeds)

Whole Tree Data:				Whole Tree One Minute Counts		Branch Data:			
Foliage Color (1):		Estimated Number of <i>Diorhabda</i> (2):		Branch Direction	Number of <i>Dior. Adults</i>	Number of <i>Dior. Larvae</i>	Number of <i>Diorhabda</i> Adults on Branch	Number of <i>Diorhabda</i> Larvae on Branch	Number of <i>Diorhabda</i> Eggs on Branch
% Green		Adults		North					
% Senescing/Yellow		Larvae		East					
% Dead Foliage		Estimated (%) Tissue Damage (3):		South					
% Dead Wood (w/o foliage)		<i>Diorhabda</i>		West					
% Regrowth		Leaf Hopper							
		Other							

Notes:
 (1) Foliage Color: %Green + % Senescing + % Dead = 100%
 (2) Estimated Number of *Diorhabda* on Tree:
 N = 0; L = 1-10; M = 11-100; H = 101-1000; V = >1000
 (3) Estimated % Tissue Damage: what % of whole tree is damaged?
 (4) Count only live bugs (not carcasses)
 (5) Count individual eggs (not egg bundles)

Comments:

SC Biocontrol Consortium Vegetation Monitoring Data Form
Pueblo, Colorado

Tree #: _____

Data Form: Nearest Neighbor (August only)

This data is collected with Trimble GPS since 2005

Date: _____

Observer: _____

Observer: _____

Recorder: _____

Time: _____

Associated Woody Vegetation:

List 3 nearest neighbors with dbh > 2.5 cm (1 inch).
 Distance and Cardinal Direction are **from monitored tree**.
 Coordinates are in Datum NAD 27, UTM Zone 13.

<u>Species name</u>		<u>Distance (m)</u>	<u>Cardinal Direction (°)</u>	<u>Northing (Y)</u>	<u>Easting (X)</u>
_____	At	_____	_____	_____	_____
_____	At	_____	_____	_____	_____
_____	At	_____	_____	_____	_____

SC Biocontrol Consortium Vegetation Monitoring Data Form
Pueblo, Colorado

Tree #: _____

Data Form: Dripline Understory Quad Data (August only)

Dripline Understory & Primary Vegetation 1 m. quadrat at outer perimeter of tree.

Date: _____

Observer: _____ **Recorder:** _____

Time: _____

Photo Numbers & Time: _____ **Whole Tree:** _____ **Quadrat:** _____ **Other(s):** _____
 (taken from the East)

_____ % Primary Vegetation Cover (within 1 m. of ground) _____ % Bare Soil _____ % Duff/Litter

Juvenile Woody Plants:

<u>List Species</u>	<u># Stems</u>	<u>% Cover</u>
<i>Baccharis glutinosa</i> (Sticky Baccharis)	_____	_____
<i>Chrysothamnus nauseosus</i> (Rabbit Brush)	_____	_____
<i>Elaeagnus angustifolia</i> (Russian Olive)	_____	_____
<i>Juniperus monosperma</i> (One Seed Juniper)	_____	_____
<i>Ribes aureum</i> (Golden Current)	_____	_____
<i>Salix spp.</i> (Willow)	_____	_____
<i>Sarcobatus vermiculatus</i> (Greasewood)	_____	_____
<i>Tamarix ramosissima</i> (Salt Cedar)	_____	_____

Herbaceous Plants:

(List all species within quadrat. Must = 100%)

<u>List Species</u>	<u>% Cover</u>
<i>Amaranthus retroflexus</i> (Redroot Pigweed)	_____
<i>Asclepias spp.</i> (Milkweed)	_____
<i>Brassica spp.</i> (Wild Mustard)	_____
<i>Bromus tectorum</i> (Downey Cheat Grass)	_____
<i>Cardaria draba</i> (Whitetop)	_____
<i>Carex spp.</i> (Field Sedge)	_____
<i>Chenopodium spp.</i> (Lambsquarter)	_____
<i>Cirsium arvense</i> (Canada Thistle)	_____
<i>Cirsium spp.</i> (Thistle)	_____
<i>Composite spp.</i>	_____
<i>Convolvulus arvensis</i> (Bindweed)	_____
<i>Distichilis spicata</i> (Salt Grass)	_____
<i>Helianthus annuus</i> (Sunflower)	_____
<i>Hordeum jubatum</i> (Foxtail)	_____
<i>Juncus balticus</i> (Baltic Rush)	_____
<i>Kochia scoparia</i> (Kochia)	_____
<i>Lactuca serriola</i> (Wild Lettuce)	_____
<i>Melilotus albus</i> (White Sweet Clover)	_____
<i>Melilotus officinalis</i> (Yellow Sweet Clover)	_____
<i>Melilotus spp.</i> (White/Yellow Sweet Clover)	_____
<i>Muhlenbergia asperifolia</i> (Scratch Grass)	_____
<i>Opuntia acanthocarpa</i> (Cholla)	_____
<i>Opuntia compressa</i> (Prickly Pear)	_____
<i>Panicum spp.</i> (Panic Type Grass)	_____
<i>Plantago major</i> (Plantain)	_____
<i>Poa spp.</i> (Kentucky Blue Grass)	_____
<i>Salsola iberica</i> (Russian Thistle/Tumbleweed)	_____
<i>Sporobulus airoides</i> (Salt Sacaton Grass)	_____
<i>Urtica dioica</i> (Nettle)	_____
Unknown (Forb)	_____
Unknown (Grass)	_____

Appendix B

**Results of Correlations between Beetle Populations and Climate Variables
Correlation Coefficients, Sample Sizes, P-values**

	#Larvae Jun	#Adult Aug	#Larvae Aug	#Adult Total	#Larvae Total	Precipt Oct-Mar	Precipt Apr-Sep	Annual Precipt	Min Temp	Max Winter Temp	First Freeze	Last Freeze	Consec days freeze	Avg DD Oct-Mar	Avg DD Apr-Sep	Year
#Adult Jun	0.9972 0.000	-0.1548 0.770	0.5048 0.307	0.0958 0.857	0.9907 0.000	-0.3273 0.527	0.2657 0.611	0.1166 0.826	0.3167 0.541	0.5158 0.295	0.4971 0.316	0.1423 0.788	0.4911 0.323	0.7439 0.090	-0.7605 0.079	-0.1494 0.778
#Larvae Jun	↘	-0.2186 0.677	0.5290 0.281	0.0308 0.954	0.9959 0.000	-0.3681 0.473	0.2455 0.639	0.0898 0.866	0.3767 0.462	0.4985 0.314	0.4532 0.367	0.1875 0.722	0.5216 0.289	0.7660 0.076	-0.7542 0.083	-0.1598 0.762
#Adult Aug		↘	-0.5179 0.293	0.9686 0.002	-0.2605 0.618	0.2029 0.700	-0.1939 0.713	-0.0949 0.858	-0.6788 0.138	0.0584 0.913	0.5168 0.294	-0.8445 0.034	-0.5774 0.230	-0.2357 0.653	0.2560 0.624	-0.2286 0.663
#Larvae Aug			↘	-0.3947 0.439	0.6033 0.205	-0.2999 0.564	0.1411 0.790	0.0274 0.959	0.0699 0.895	-0.3857 0.450	-0.4428 0.379	0.8132 0.049	0.0135 0.980	0.6041 0.204	-0.8332 0.039	0.1213 0.819
#Adult Total				↘	-0.0130 0.981	0.1220 0.818	-0.1284 0.808	-0.0663 0.901	-0.6042 0.204	0.1887 0.720	0.6458 0.166	-0.8150 0.048	-0.4581 0.361	-0.0502 0.925	0.0665 0.901	0.2680 0.608
#Larvae Total					↘	-0.3778 0.460	0.2457 0.639	0.0873 0.869	0.3614 0.482	0.4275 0.398	0.3788 0.459	0.2626 0.615	0.4916 0.322	0.7840 0.065	-0.7973 0.058	-0.1373 0.795

Correlation

P-value

Highlighted value = significant P at alpha=0.05

Sample size n=6

Appendix C

**Results of Correlations between Variables
Correlation Coefficients, Sample Sizes, P-values**

	#Larvae	%Damage	%Wood w/o foliage	%Green foliage	%Dead foliage	%Senesced foliage	%Regrowth	%Trees w/ flowers	Cross area	LAI	%Native grass	%Intro forbs	Year	Precipt.
#Adults	0.4824 309 0.000	0.7427 309 0.000	0.1550 309 0.007	-0.5061 309 0.000	0.6750 309 0.000	0.3149 309 0.315	0.3788 309 0.000	0.0579 7 0.902	0.1514 309 0.008	-0.2890 154 0.000	-0.0917 309 0.108	0.1628 309 0.004	0.5097 309 0.000	-0.1062 7 0.821
#Larvae	↘	0.4612 309 0.000	0.2859 309 0.000	-0.2656 309 0.000	0.3655 309 0.000	-0.0557 309 0.329	0.5902 309 0.000	-0.6654 7 0.103	0.0906 309 0.112	0.2614 154 0.001	-0.1317 309 0.021	0.1654 309 0.004	0.3750 309 0.000	0.0560 7 0.0905
%Damage	↘	↘	0.3018 309 0.000	-0.6669 309 0.000	0.8406 309 0.000	-0.2376 309 0.000	0.4668 309 0.000	-0.4509 7 0.310	0.7427 309 0.000	-0.5164 154 0.000	-0.1644 309 0.004	0.2228 309 0.000	0.6528 309 0.000	0.2236 7 0.630
%Wood w/o foliage			↘	-0.2489 309 0.000	0.1841 309 0.001	-0.0070 309 0.902	0.3561 309 0.000	0.6010 7 0.154	-0.0065 309 0.909	-0.3050 154 0.706	-0.2471 309 0.000	0.2425 309 0.000	0.4524 309 0.000	0.5516 7 0.199
%Green foliage				↘	-0.7742 309 0.000	0.0026 309 0.963	-0.2298 309 0.000	0.6180 7 0.139	0.1467 309 0.010	0.5446 154 0.000	0.0400 309 0.483	-0.1032 309 0.070	-0.3780 309 0.000	-0.0808 7 0.863
%Dead foliage					↘	-0.0846 309 0.137	0.3618 309 0.000	-0.4504 7 0.311	-0.0077 309 0.892	-0.4938 154 0.000	-0.0730 309 0.200	0.1192 309 0.036	0.5365 309 0.000	0.0265 7 0.965
%Senesced foliage						↘	-0.0941 7 0.099	0.4775 309 0.279	0.2520 309 0.000	0.4507 154 0.000	0.0440 309 0.440	-0.0764 309 0.180	0.0096 309 0.866	-0.5174 7 0.234
%Regrowth							↘	-0.2017 7 0.665	0.1261 309 0.027	0.2007 154 0.013	-0.1637 309 0.004	0.1957 309 0.001	0.5324 309 0.000	0.1381 7 0.768
%Trees w/ flowers								↘	0.6887 7 0.087	0.2920 4 0.708	0.4242 7 0.343	-0.4594 7 0.300	-0.2399 7 0.604	-0.0642 7 0.891
Cross area									↘	0.5759 154 0.000	-0.0535 309 0.348	0.0034 309 0.952	0.1059 309 0.063	0.0627 7 0.894
LAI										↘	-0.2116 154 0.001	0.1439 154 0.075	0.1594 154 0.049	0.0742 4 0.926
%Native grass											↘	-0.8833 309 0.000	-0.2260 309 0.000	-0.6522 7 0.112
%Intro forbs												↘	0.2769 309 0.000	0.6542 7 0.111
Year													↘	0.6286 7 0.131

Correlation

Sample size

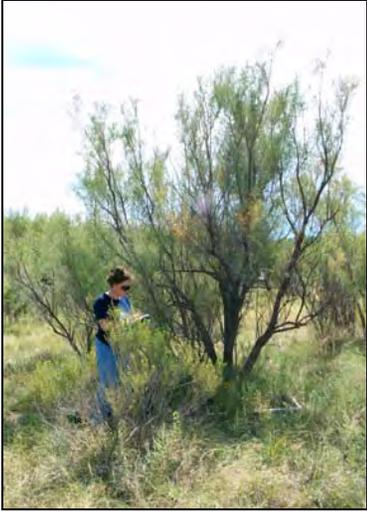
P-value

Highlighted value=significant P at alpha=0.05

Appendix D

**Photo Comparisons of Original 100 Trees
2001 vs. 2007**

Tree #1



2001



2007

Tree #2



2001



2007

Tree #3



2001



2007

Tree #4



2001



2007

Tree #5



2001



2007

Tree #6



2001



2007

Tree #7



2001



2007

Tree #8

**No photo available
2001**



2007

Tree #9



2001



2007

Tree #10



2001



2007

Tree #11



2001



2007

Tree #12



2001



2007

Tree #13



2001



2007

Tree#14



2001



2007

Tree #15



2002



2007

Tree #16



2003



2007

Tree #17



2001



2007

Tree #18



2001



2007

Tree #19



2001



2007

Tree #20



2002



2007

Tree #21



2001



2007

Tree #22



2001



2007

Tree #23



2001



2007

Tree #24



2001

Tree #25



2007



2001



2007

Tree #26



2001



2007

Tree #27



2001



2007

Tree #28



2001



2007

Tree #29



2001



2007

Tree #30

**No photo available
2001**



2007

Tree #31

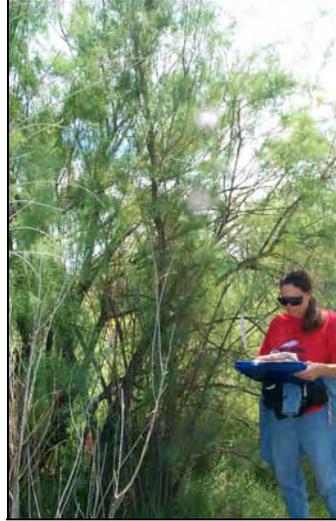


2001



2007

Tree #32



2001



2007

Tree #33



2001



2007

Tree #34



2001



2007

Tree#35



2001



2007

Tree #36



2001



2007

Tree #37



2001



2007

Tree #38



2001



2007

Tree #39



2001



2007

Tree #40



2002



2007

Tree #41



2001



2007

Tree #42



2001



2007

Tree #43



2001



2007

Tree #44



2001



2007

Tree #45



2001



2007

Tree #46



2001



2007

Tree #47



2001



2007

Tree #48



2001



2007

Tree #49



2001



2007

Tree #50



2001



2007

Tree #51



2001



2007

Tree #52



2001



2007

Tree #53



2001



2007

Tree #54



2001



2007

Tree #55



2001



2007

Tree #56



2001

**Tree removed
2007**

Tree #57



2001

**Tree removed
2007**

Tree #58



2001



2007

Tree #59



2001



2007

Tree #60



2001



2007

Tree #61



2001



2007

Tree #62



2001



2007

Tree #63



2001



2007

Tree #64



2001



2007

Tree #65



2001



2007

Tree #66



2001

2007
Tree removed

Tree #67



2001



2007

Tree #68



2001



2007

Tree #69



2001



2007

Tree #70



2001

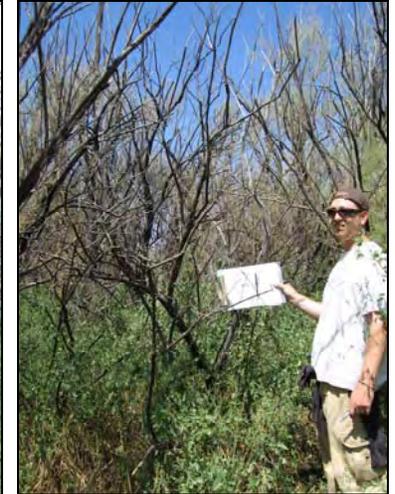


2007

Tree #71



2001



2007

Tree #72



2001



2007

Tree #73

**No photo available
2001**



2007

Tree #74



2001



2007

Tree #75



2001



2007

Tree #76



2001



2007

Tree #77



2001



2007

Tree #78



2001



2007

Tree #79



2001



2007

Tree #80



2001



2007

Tree #81



2001



2007

Tree #82



2001



2007

Tree #83



2001



2007

Tree #84



2001



2007

Tree #85



2001



2007

Tree #86



2001



2007

Tree #87



2001



2007

Tree #88



2001



2007

Tree #89



2001



2007

Tree #90



2001



2007

Tree #91



2001



2007

Tree #92



2001



2007

Tree #93



2001



2007

Tree #94



2001



2007

Tree #95



2001



2007

Tree #96



2001



2007

Tree #97



2001



2007

Tree #98



2001



2007

Tree #99



2001



2007

Tree #100



2001



2007

Appendix E

**Photo Comparisons of Associated Understory Quadrats
2001 vs. 2007**

Quadrat 2



2001



2007

Quadrat 4



2001



2007

Quadrat 7



2001



2007

Quadrat 9



2001



2007

Quadrat 10



2001



2007

Quadrat 11



2001



2007

Quadrat 13



2001



2007

Quadrat 18



2001



2007

Quadrat 24



2001



2007

Quadrat 27



2001



2007

Quadrat 31



2001



2007

Quadrat 35



2001



2007

Quadrat 36



2001



2007

Quadrat 38



2001



2007

Quadrat 41



2001



2007

Quadrat 44



2001



2007

Quadrat 45



2001



2007

Quadrat 48



2001



2007

Quadrat 51



2001



2007

Quadrat 54



2001



2007

Quadrat 58



2001



2007

Quadrat 59



2001



2007

Quadrat 63



2001



2007

Quadrat 69



2001



2007

Quadrat 74



2001



2007

Quadrat 75



2001



2007

Quadrat 77



2001



2007

Quadrat 78



2001



2007

Quadrat 81



2001



2007

Quadrat 82



2001



2007

Quadrat 84



2001



2007

Quadrat 89



2001



2007

Quadrat 91



2001



2007

Quadrat 92



2001



2007

Quadrat 94



2001



2007

Quadrat 96



2001



2007

Quadrat 99



2001



2007

Quadrat 100



2001



2007

Appendix F

**Photo Comparisons of Photo Stations
2004 to 2007**

Photo Station 1



2004



2005



2006



2007

Photo Station 2



2004



2005



2009



2007

Photo Station 3



2004



2005



2006



2007

Photo Station 4



2004



2005



2006



2007

Photo Station 5



2004



2005



2006



2007

Photo Station 6



2004



2005



2006



2007

Photo Station 7



2004



2005



2006



2007

Photo Station 8



2005



2006



2007

Photo Station 9



2005



2006

2007 No photo available

Photo Station 10



2005



2006



2007

Photo Station 11



2005



2006



2007

Photo Station 12



2005



2006



2007

Photo Station 13



2005



2006



2007

Photo Station 14A



2005



2006



2007

Photo Station 14B



2005



2006



2007

Photo Station 14C



2005



2006



2007

Photo Station 14D



2005



2006

2007
No Photo Available