A Synopsis of Saltcedar Biological Control Using *Diorhabda elongata* 
Along the Canadian River in Texas, 2002-2007

G. J. Michels, V. A. Carney, E. N. Jones, J. B. Bible 
Texas AgriLife Research 
Bushland, Texas 79012

In 2002, a cooperative saltcedar biological control program commenced involving Texas AgriLife Research in Amarillo, the US Bureau of Reclamation, the Canadian River Municipal Water Authority and the National Parks Service, targeting *Tamarix* infestations along the Canadian River and in the Lake Meredith area. As Lake Meredith supplies over 77,000 acre/feet of freshwater to Amarillo and surrounding municipalities, saltcedar eradication is a key priority in this region.

Eggs from a strain of *D. elongata* originating in Posidi, (northern) Greece were obtained in 2004 and released into cages near Lake Meredith to facilitate their establishment. There have been no previous studies on the development, population growth and effect of this particular strain of saltcedar leaf beetle in Texas, although a strain more suited for southern climates has been studied in the Temple area.

**Program Activities in 2004**

The climatic conditions at this Lake Meredith-area study site were atypical in 2004, characterized by an overall cooler growing season and an abundance of moisture, with flooding conditions frequently throughout the summer and early fall. Of particular concern to this biocontrol program were a cold period immediately following the implementation of the saltcedar beetle release project (as it relates to hatch and establishment of the biocontrol agent), repeated flooding of the survey areas impeding access to the site for insect monitoring, and cool temperatures, rainy conditions and early senescence of host tree materials in late summer, which have the combined potential to negatively affect the ability of these biocontrol agents to prepare for overwintering. Despite their exposure to abnormal climatic conditions this year, the saltcedar biocontrol beetles (*Diorhabda elongata*) established and achieved high numbers and showed promising dispersal capabilities in one season of release and monitoring.

**Impact Survey and Long-term Vegetation Monitoring in Release Area**

Following vegetation and insect monitoring protocols adapted from those used by the Consortium for the Biological Control of Saltcedar, selected trees at 50, 100 and 200 m from the initial salt cedar beetle release site were marked and surveyed twice in 2004. The primary objective of this vegetation monitoring is to provide baseline data on the extent and impact of saltcedar in this region of Lake Meredith. Tree characteristics and neighboring vegetation were quantified this season in May, and again in September. Forty saltcedar trees (10 at each 50 and 100 m, 20 at a 200 m radius) were evaluated for height, bulk, canopy cover, potential seed production, foliage quality, and seedling abundance. The densities of saltcedar trees in the study area were indirectly measured by a nearest-neighbor analysis. Characteristics of these selected trees will be compared through successive years in order to quantify impact of the biocontrol.
agent, *D. elongata*, as it establishes and defoliates the saltcedar in this area. This season’s data collection provided a rare opportunity in biological control to collect such extensive baseline vegetation data. Future impact of *D. elongata* defoliation on the quality and vigor of saltcedar trees can now be quantified with greater reliability.

The 2004 program also enlisted the services of contractor, J. R. Bell, a vegetation expert in the Lake Meredith area, for assistance with plant species identification and community ecology. His co-operation with this project enabled us to gain a baseline snapshot of the plant community composition in our study area. Successful control of saltcedar by *D. elongata* should result in a positive shift of this vegetation community toward greater colonization by native and other plant species identified this season.

Biocontrol Agent Release, Study and Monitoring

On April 21, 2004, two thousand eggs of a northern Greece strain of *D. elongata* were released on saltcedar foliage at a study site near Lake Meredith, TX within an enclosed 12’ x 12’ x 6’ mesh tent. Mesh sleeves were used to secure the egg masses into selected foliage and to exclude larger predators, although ants and small parasitoids still had access to the eggs. The bags were checked biweekly until large larvae (2nd and 3rd instars) were observed, at which point the mesh protection was removed to facilitate pupation of the beetles underground. A hatch rate of approximately 30% was observed from the original egg release. It is estimated that approximately 150 adult *D. elongata* resulted in early June from the initial release of 200 eggs (8% survival within the first generation), a rather typical survivorship rate for this type of insect. During this first generation, evidence of feeding could be seen only by close examination of the saltcedar leaflets.

Evidence of saltcedar beetle defoliation on trees within the tent enclosure was visible as a browning of foliage, as individual leaflets died out throughout June and July. The population of *D. elongata* increased over 10-fold from the first to second generation and the second generation was completed by mid-July. Between 1500 and 2000 adults were observed the week of July 20, at which time over 500 adults were released from the tent enclosure, as fresh saltcedar foliage was becoming a rare commodity. On July 26, the tent enclosure was removed to allow adult beetle dispersal and prevent starvation of third generation offspring beginning to feed in the tent.

A preliminary reproduction, development and longevity study commenced on July 20 using newly-emerged, second generation adult *D. elongata*. Ten mating pairs of virgin female and newly emerged male beetles were placed into mesh sleeves around foliage within the vicinity of the initial biocontrol agent release. Biweekly counts of oviposition and larval survival were performed, with the adult pairs being moved to fresh foliage during each count. The second generation adults laid between 17 and 97 eggs over a lifespan of 9-42 days. An average of 40 days was observed for the longevity of each of the three *D. elongata* generations in 2004. This translates into a rate of oviposition of 1-4.5 eggs laid daily during a female beetle’s lifespan. More detailed studies are planned for subsequent years but from this initial data, it is predicted that each female *D. elongata* surviving the average 40 days can produce up to 200 eggs in her lifespan. We were unable to calculate offspring development rates from this study, as extreme mortality levels caused by ants, spiders and other generalist predators were observed, particularly on the egg and neonate stages.

The saltcedar-defoliating beetles are not expected to disperse far beyond the initial area of release in the 2004, or even 2005 season. Localized damage to saltcedar trees is predicted, if
the beetles successfully overwinter. However, one anecdotal observation of *D. elongata* was made during the vegetation surveying in early September. A single adult of the 3rd generation was noted on a marked tree at 100m from the initial point of release. Close monitoring of all trees marked for long-term vegetation study for beetle impact is, therefore, critical in 2005.

**Program Activities in 2005**

Beginning in March 2005, monitoring was initiated at the study site to detect emergence of the beetles. While the beetles had been released into tents and were thus fully contained during the beginning of the 2004 season, the tent sites were opened for an extended period in the fall of 2004 and again in 2005 to allow for natural establishment of the population to the surrounding area. Overwintering success was confirmed on 26 April 2005 when 7 male beetles were located actively feeding on saltcedar. Weekly surveys of the tent sites and surrounding areas continued to yield adult beetles indicating that the beetles were effectively emerging from a period of diapause. By 7 June, multiple sites around the initial release points were showing light herbivory damage and defoliation indicative of activity by the saltcedar-feeding beetle. Reproduction was corroborated with the sighting of numerous larvae in various stages of development throughout the site and extending in a 6 meter radius from the release site. These sites continued to be surveyed throughout the summer resulting in the verification of three generations of beetles. The development rate of *D. elongata* observed in 2005 corresponded to observations made the previous season. On 30 June, 112 adult beetles obtained from a rearing facility in California were released into the original tent site to add genetic diversity to the established population. By 9 August, reproduction in the release tent had produced large numbers of larvae with resultant heavy damage to the saltcedar trees contained within the tent.

Four new containment tent sites were established to accommodate a redistribution of these beetles and larvae to increase their overwintering and distribution success. One hundred adult and larval-stage beetles were transferred to each of the new tent sites. Within two weeks after the transfer, the saltcedar inside each of these tents began to show signs of senescence and the beetles were no longer seen feeding on the leaves. This follows a similar pattern that was noted during the 2004 season, indicating that this generation was preparing for overwintering in the soil.

Vegetation surveys of the 40 sentinel saltcedar that had been selected in 2004 were conducted in June and again in September 2005. Data for each of these trees was recorded relative to growth and seed production. In addition, information regarding the soil type, percent ground cover, vegetative abundance and types of woody plants in proximity to these saltcedar was documented. This data will be used in a comparative study to assess both the impact and extent of the saltcedar in the area, along with efficacy of the biocontrol agents in future years. These saltcedar are located in concentric circles around the release site at distances of 50, 100 and 200 meters. During the June 2005 survey, larvae were located at two of the trees corresponding to 50 meters from the initial release site, and an adult beetle was found feeding on a tree at the 200 meter distance. Another tree at the 200 meter distance showed extensive defoliation consistent with *D. elongata* feeding. A single adult beetle was observed 24 August at the trailhead to the release area, approximately 1 km away from the original release site. These
findings confirm that the beetles have successfully dispersed significant distances from the initial release site.

A ground beetle species study was initiated to determine their diversity and density within the saltcedar-infested area around Lake Meredith. This study serves as a qualitative means to indicate changes in the ecosystem, particularly as saltcedar is reduced by the beetle biocontrol agents. The sampling sites were established in 3 distinct habitats with 9 collection points in each area. The first sampling area was located east of the release site in saltcedar that had been subjected to a controlled burn in April 2005, the second site was located in a stand of saltcedar near the release site, and the third sampling site was located north of the release site in an open area with sparse grasses and brush. The samples were collected on a biweekly basis and will serve as a baseline indicator of ground insect species richness prior to saltcedar decline. Population levels and species composition of ground beetles are expected to be altered in subsequent years as saltcedar, reduced by the biological control agents, is replaced by a more natural plant and grass community.

**Program Activities in 2006**

A significant expansion of the saltcedar biocontrol program occurred in 2006. As mentioned above, in addition to the monitoring efforts at Lake Meredith, we began a new release program on privately-owned land north of Borger, TX, expanded the number of beetle ecotypes in our study and conducted lab-based research in Bushland. A detailed update on the work to date at each location follows.

**Lake Meredith**

Weekly surveys to detect established biological control agent, *Diorhabda elongata*, began in March. The first overwintering beetles, approximately 60 adults, were observed on April 12 inside the initial release cage (i.e. cage #1). Shortly after the second year of overwintering survival was confirmed, we began observing individuals outside the release cages in both adult (May 3) and larval stages (June 6). Extensive damage to saltcedar foliage occurred in two of the four supplementary release cages erected in 2005 (i.e. cages #3 & 5) by June 2006, coincident with the emergence of the second spring generation of beetles. Saltcedar damage by *D. elongata* increased within both cages until July 13, at which time, the mesh screening on cage #5 had to be removed in order to allow beetles to escape, as 100% of the foliage inside the cage was damaged. Two more generations of *D. elongata* occurred through July and August. A few adult beetles were observed clinging to saltcedar trees as late as October 26, one to two months later than the 2004-2005 populations were observed to have entered winter diapause.

Despite successful overwintering in 2005-2006 and promising beetle numbers observed early this season, we did not find widespread damage to saltcedar trees in 2006, nor were many beetles recovered outside the release cages as the summer progressed. Previous years’ observations indicate that these highly mobile beetles disperse readily as they are released into open saltcedar stands, making it increasingly difficult to quantify the population levels at Lake Meredith. Preliminary evaluation of a new monitoring technique employing insect pheromones and plant volatile compounds was performed on June 6. Although it has been utilized successfully to estimate numbers of live beetles at other release locations in the USA where populations of *D. elongata* are extremely high, we were not successful in attracting and retaining
beetles in our selected monitoring zones. The method will be re-applied with the addition of sticky traps in subsequent years if beetle populations have increased to sufficient levels that mortality due to trap catch will not harm established beetle numbers.

To address the waning numbers of beetles found during weekly surveys, we made supplementary releases of *D. elongata* from a small, lab-reared colony kept at the Experiment Station in Bushland, TX (originating from Posidi, Greece). Beetle releases were made on July 17 (~200 individuals placed into the initial release cage), July 20 (60 additional beetles released outside the initial release cage) and August 31 (80 adult beetles released into a nearby saltcedar plot, burned in 2005).

Vegetation surveys, similar to those conducted in 2004 and 2005, were performed on June 19 with the cooperation of local vegetation expert J. R. Bell. Plant community and species identifications were made by Mr. Bell and 40 sentinel saltcedar trees were measured for plant growth, damage levels, foliage quality etc. These data are part of a long-term study on the shift in plant community as biological control agents repeatedly damage saltcedar.

For the second year, ground beetles were collected at Lake Meredith using pitfall traps and identified by an insect taxonomist. Ground beetles were chosen as a taxonomic group of study as they are large, easily collected predators, whose presence in a habitat can be used to indicate the amount of available insect prey, habitat complexity and changes within the immediate environment. Comparisons of ground beetle species diversity and abundance will be made over several years and across different areas within the saltcedar study site: open prairie floodplain, dense saltcedar stands, saltcedar burned in early 2005. Changes in ground beetle species composition should also reflect the decline of saltcedar as biological control agents stress and kill trees.

Ground beetle trapping was conducted between March and December in both 2005 and 2006. We collected a total of 2705 ground beetles within 27 pitfall traps (nine traps in each collection area) in 2005. These were classified into 33 separate species. Immediate differences in species composition were observed between the collection areas with trees (i.e. the dense saltcedar stand, burned saltcedar area) and the open flats. Three species were observed only in the open prairie flats, one species occurred in both treed areas, four species were unique to the dense saltcedar habitat and 13 species appeared to be restricted to the newly burned saltcedar area. The burned saltcedar habitat is interesting, as it contained the vast majority of beetles collected during 2005 (1790 individuals) in addition to having the largest number of unique species. We are awaiting identification on approximately 1500 specimens collected in 2006 in order to statistically analyze trend information.

Johnson Ranch Site (north of Borger, TX)

On April 27, we received a shipment of approximately 200 *D. elongata* eggs from a USDA-ARS Temple cooperator and released them into cages on private land north of Borger, TX. The beetles originated from a collection point in Uzbekistan and have some morphological and genetic differences from the Lake Meredith (Posidi, Greece source) population. Additional releases of eggs and adults into cages on this property yielded excellent population levels of the Uzbek-strain beetles (from approximately 300 initial insects) in their first season. We observed 4 additional generations of *D. elongata* (Uzbek) at this site and beetles remained actively feeding well into October. In one of the 12’ x 6’ release cages, beetle populations exploded between the 2nd and 4th generations, completely defoliating the available saltcedar.
Permission was granted in early summer to release this beetle ecotype into the environment, so the cage mesh was removed and larval instars transferred to nearby saltcedar trees. As late as October 26, we observed adult beetles returning to the damaged trees from the cage and consuming all available saltcedar re-growth within the cage area. Production from the cages on this property was conservatively estimated to be 5000 beetles.

In August of 2006, we began work in conjunction with a multi-state research initiative to experimentally test the predicted ranges and survival potential of five ecotypes of *D. elongata* at several locations throughout the southwestern US (i.e. the Latitudinal Gradient study). Working with beetles from Posidi and Crete, Greece, Uzbekistan, Tunisia and Turpan, China, we housed each ecotype within sleeve cages on saltcedar branches inside a 12’ x 6’ cage to maintain isolated beetle populations. Between August 31 and October 26, we evaluated development rate of each ecotype in the Texas Panhandle and looked for signs of overwintering diapause readiness. The beetles completed one generation in this time and a partial second generation before the senescence of saltcedar and onset of cold weather prevented completion to adulthood. We continue to evaluate these beetles over the 2006-2007 winter in diapause containers. Survival of each ecotype will be evaluated in early spring and the entire experiment is scheduled to be conducted throughout the summer of 2007.

**Texas AgriLife Research Life History Studies**

Three saltcedar biocontrol agent experiments were conducted in late 2006 at the Texas AgriLife Research lab in Bushland. Comparative development and mortality studies were performed using Posidi- and Uzbekistan-source *D. elongata*, reared under Texas field conditions. Initial results indicate that the Uzbek beetles have a faster development rate than the Posidi ecotype. Detailed life table studies have begun for all five beetle ecotypes involved in the Latitudinal Gradient study. We are exposing individual neonate larvae to saltcedar in one of three controlled temperature/daylength regimes to simulate seasonal differences. These data will be compared across beetle species. We hope to better understand the climatic restrictions and requirements of these biocontrol agents in order to maximize their efficacy.

In conjunction with the Latitudinal Gradient study, we are currently housing each of five beetle ecotypes in overwintering containers at Texas AgriLife Research-Bushland. Diapause-ready adult beetles from Fukang, Crete, Tunisia, Turpan and Uzbekistan were buried in the soil in late fall to simulate natural overwintering conditions. Winter survival will be assessed in early spring 2007, coincident with saltcedar bud break in the area. Winter temperatures are also being monitored to allow for year-to-year comparisons of Texas Panhandle field conditions.

**Program Activities in 2007**

**Lake Meredith**

A new release site was established this year on the north side of Lake Meredith near Plum Creek. In May of 2007, 130 Posidi beetles were released on a caged saltcedar tree at Plum Creek. The beetles reproduced quickly and soon thousands of larvae were observed in the release cage. The screen cage was removed on June 27th due to defoliation of the tree and to allow the beetles to disperse to nearby saltcedar. Biweekly monitoring of beetle movement revealed that adult
Diorhabda had established in the surrounding trees and these saltcedars were showing signs of damage. To increase population numbers, a sleeve containing six females and one male was placed on a neighboring tree on June 20th, then checked on June 27th. Twenty eggs masses were produced and released with the rest of the population. This site is going to be very interesting to monitor over the next couple of years as the defoliation continues. Plum Creek is different than other sites at Lake Meredith in terms of saltcedar tree density. The stand is so thick that one can barely walk through them. It is an ideal location for biological control because the density of plant material directly improves probability of successful establishment and impact.

At the Mullinaw Crossing site, a small population was observed this year throughout the season. About 50 adults and larvae were seen inside the original release cage at peak of this population; these numbers were very low, however the population has sustained itself since 2004 and managed to survive the Texas Panhandle’s variable winter conditions.

Again this year, ground beetles were collected at the Mullinaw Crossing and Johnson Ranch sites to be used as environmental indicators. As the biological control beetles stress and kill saltcedar trees in those areas, the ground beetle abundance and diversity should reflect the changes in the ecosystem. Based on the collections made in 2005 and 2006, we are already observing significant differences in ground beetle community structure and abundance between heavily infested saltcedar areas versus open floodplain and treated saltcedar areas. The highest abundance and diversity of ground beetles has been found both years within a patch of saltcedar burned in early 2005. We will continue to monitor the ground beetles over the next several years as Diorhabda defoliation becomes more widespread and begins to impact saltcedar stands.

Vegetation surveys were conducted again this year to continue to provide baseline information about the saltcedar trees at Mullinaw Crossing and Johnson Ranch prior to Diorhabda impact. The native plant community was also surveyed by Texas AgriLife Research staff and contractor J. R. Bell. This data will be used to document the changes in plant community structure as saltcedar trees are eliminated from the ecosystem.

Johnson Ranch (north of Borger, TX)

On the Canadian River north of Borger TX, Diorhabda beetles from Uzbekistan were established last year and an open release was conducted at the Johnson Ranch site. The overwintered population emerged successfully in the spring of 2007 in release cages. Mid-summer, there was an “explosion” of beetles inside the cage so the screen cage was removed to release about 200 adult beetles into the open. Defoliation on the caged saltcedar tree was substantial and many larvae were seen in addition to the adult beetles. The beetles were monitored throughout the season for signs of establishment on neighboring trees. Also, two new release cages were set up this spring and three releases made, totaling 290 Uzbekistan beetles released at Johnson Ranch in 2007.

Near the Diorhabda release cages at Johnson Ranch, biweekly spider surveys were conducted to monitor the abundance of one of potential predators of the leaf beetles. Specimens and study results were sent to New Mexico State University, where lab tests are being run to determine which spiders are potentially harmful to the beetles as not all spiders will actively feed on Diorhabda. This information will help to determine which sites are realistic for biological control and improve the success of future bio-agent releases.

Palo Duro Canyon
In cooperation with Palo Duro State Park, a new program was initiated this year in an effort to control saltcedar in the park. However, due to severe flooding in the park, the beetles were unable to establish. Uzbekistan beetles were released onto saltcedar in a 6’ x 12’ cage on July 16th and checked biweekly. When technicians visited the site on August 16th, the cage had flood lines five feet high. One beetle was found and the cage was removed. Next year, we will seek out a new location on higher ground and more releases will be made.

**Diorhabda Rearing Cages at Bushland**

Outside the entomology laboratory in Bushland, four 20’ x 12’ cages have been erected and filled with greenhouse-propagated saltcedar. Beetles from Uzbekistan were placed in these cages to rear massive populations of *Diorhabda* beetles. Several hundred beetles were observed in the cage by late July. In late September, five females from the population were dissected looking for signs of diapause, a physical state similar to dormancy. The results of the dissections showed that the insects were entering diapause for the winter at the appropriate time for this geographic area. This population will be used to produce larger numbers of insects for release in the spring.

**Other Activities**

In cooperation with scientist around the nation, we participated in a Latitudinal Gradient Study to determine which *Diorhabda* beetle is the best suited for the Texas Panhandle. To date, there are several ecotypes of *Diorhabda* beetles that require more detailed study into their physiological and ecological adaptations to various locations in the US. They include the Uzbek and Posidi, Greece beetles currently being used in this area, along with beetles originating from Crete, Greece and Fukang and Turpan, China. The purpose of the experiment is to determine how many generations each ecotype can have at different latitudes and to study when they enter reproductive diapause at the appropriate time to survive winter conditions in the various study areas in order to recommend which ecotype will be best suited for introduction in different regions. The basic approach is to rear the beetles from eggs to adults in the field. When new adults emerge (F1, F2 generations etc), the beetles will be tested in the field for egg laying capacity and specimens collected to check their reproductive status. Larval production is also quantified for each generation of beetles. We will continue to participate in this study for the next few years.

A life history study of the *Diorhabda* ecotypes were completed this year. This study compared development and mortality rates of the different beetle ecotypes under controlled temperature and day length regimes using incubators. This research will allow scientists to better predict the minimum development thresholds for different beetle ecotypes and indicate which ecotypes will survive at the various latitudes.