Clean Water Act Section 319(h) Nonpoint Source Pollution Control Program Projects

Biological Control of Saltcedar: Mathematical Modeling of Dispersal of the Leaf Beetle, *Diorhabda elongata*

TSSWCB Project # 04-15
Revision 1

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

Prepared by

United States Department of Agriculture
Agricultural Research Service
and
Texas Agricultural Experiment Station

Effective Period: 3 Years from May 2005 to April 2008

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Section A1: Approval Sheet

Quality Assurance Project Plan for Project. Biological Control of Saltcedar: Mathematical Modeling of Dispersal of the Leaf Beetle, *Diorhabda elongata*

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List of Acronyms and Abbreviations

APHIS – USDA Animal and Plant Health Inspection Service
ARS – Agricultural Research Service (USDA)
BC/SC – Biological Control of Saltcedar
BC/SCC – Biological Control of Saltcedar Consortium
BC/SCC, TX, NM, MX – Biological Control of Saltcedar Consortium: Texas, New Mexico, Mexico Section
CAR – Corrective Action Report
COC – Chain of Custody
CRMWD – Colorado River Municipal Water District
EDAS – Ecological Data Applications System
EPA – Environmental Protection Agency
FS – USDA Forest Service
GSWRL – Grassland, Soil and Water Research Laboratory (USDA-ARS, Temple, TX)
NMSU – New Mexico State University, Las Cruces, NM
NRCS – USDA Natural Resource Conservation Service
QA
QAPP – Quality Assurance Project Plan
SEL – Systematic Entomological Laboratory (USDA-ARS, Beltsville, MD)
SOP – Standard Operating Procedure
TAES – Texas Agricultural Experiment Station
TAMU – Texas A&M University
TCE – Texas Cooperative Extension
TSSWCB – Texas State Soil and Water Conservation Board
USDA – United States Department of Agriculture
USDA-ARS – USDA-Agricultural Research Service
USDA-NRCS – USDA-Natural Resources Conservation Service
USDI-BOR – U.S. Department of Interior-Bureau of Reclamation
USEPA

Section A3: Distribution List

Organizations, and individuals within, which will receive copies of the approved QAPP and any subsequent revisions include:

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Section A4: Project/Task Organization

The following is a list of individuals and organizations participating in the project with their specific roles and responsibilities:

USEPA – United States Environmental Protection Agency (USEPA), Region VI, Dallas. Provides project overview at the Federal level.

Ellen Caldwell, USEPA Chief, State/Tribal Programs Section
Responsible for overall performance and direction of the project at the Federal level.
Approves the final products and deliverables.

TSSWCB – Texas State Soil and Water Conservation Board (TSSWCB), Temple, Texas. Project Lead.
Pamela Casebolt, TSSWCB Project Manager
Donna Long, TSSWCB Quality Assurance Officer

Project Implementation Personnel

C. Jack DeLoach, Ph.D. – Project Manager/Quality Assurance Manager (ARS) – Plans research and field demonstrations. Oversees the research execution and data collection, supervises personnel, authorizes expenditures, writes reports to TSSWCB, maintains the official, approved Project Research Plan. Prepares QAPP and forwards to TSSWCB for approval. Assures that experiments and demonstrations are conducted and data is collected according to approved QAPP. Maintains the official, approved QA Project Plan.

Joaquin Sanabria, Ph.D. – Assistant Research Scientist, TAES, Temple – Constructs the model, modifies and validates it from field data. Analyzes collected data with the purpose of identifying biotic and abiotic factors associated with *Diorhabda* establishment and dispersal.

Allen Knutson, Ph.D. – Entomologist, TAES, Dallas – Conducts independent research on dispersal of *Diorhabda* beetles and effects of beetle defoliation on carbohydrate reserves of saltcedar plants; cooperates with ARS in releases and monitoring of *Diorhabda* beetles.

Post Doctoral Scientist (Vacant) – Project Biologist – Conducts research and field demonstrations on dispersal, field ecology and field biology of the *Diorhabda* beetles, cooperates with research projects of TAES scientist Joaquin Sanabria.

James Tracy, M.S. – ARS Project Lead Technician – Sets up experiments and demonstrations in the field, performs monitoring of *Diorhabda* beetles, vegetation and acquisition of climatic data described in Research Plan; supervises daily work activities and is assisted by temporary personnel.

Tom Robbins, B.S. – ARS Project Technician – Works together with Technician Tracy in collecting data on monitoring of *Diorhabda* beetle dispersal, vegetation surveys, and acquisition of climatic data.

Jeremy Hudgeons, M.S. Graduate Assistant of Dr. Knutson, Texas A&M University, Department of Entomology.

Okla Thornton, M.S. – Ecologist, Colorado River Municipal Water District – Assists in site location and planning of beetle, plant and wildlife monitoring; liaison with local landowners and water district operations.

Jeff Knight, Chief Entomology Section, Nevada Department of Agriculture, Reno, NV – Authorizes sampling and use of field data at the Lovelock and Schurz, NV release sites of *Diorhabda* beetles.

Raymond I. Carruthers, Research Leader, ARS Exotic and Invasive Weed Research, Albany, CA - Authorizes sampling and use of field data and aerial remote sensing imagery at the Lovelock and Schurz, NV release sites of *Diorhabda* beetles.

Fred Nibling, Research Botanist, USDI, Bureau of Reclamation, Denver, CO – Authorizes sampling and use of field data and aerial remote sensing imagery at the Pueblo, CO release sites of *Diorhabda* beetles.

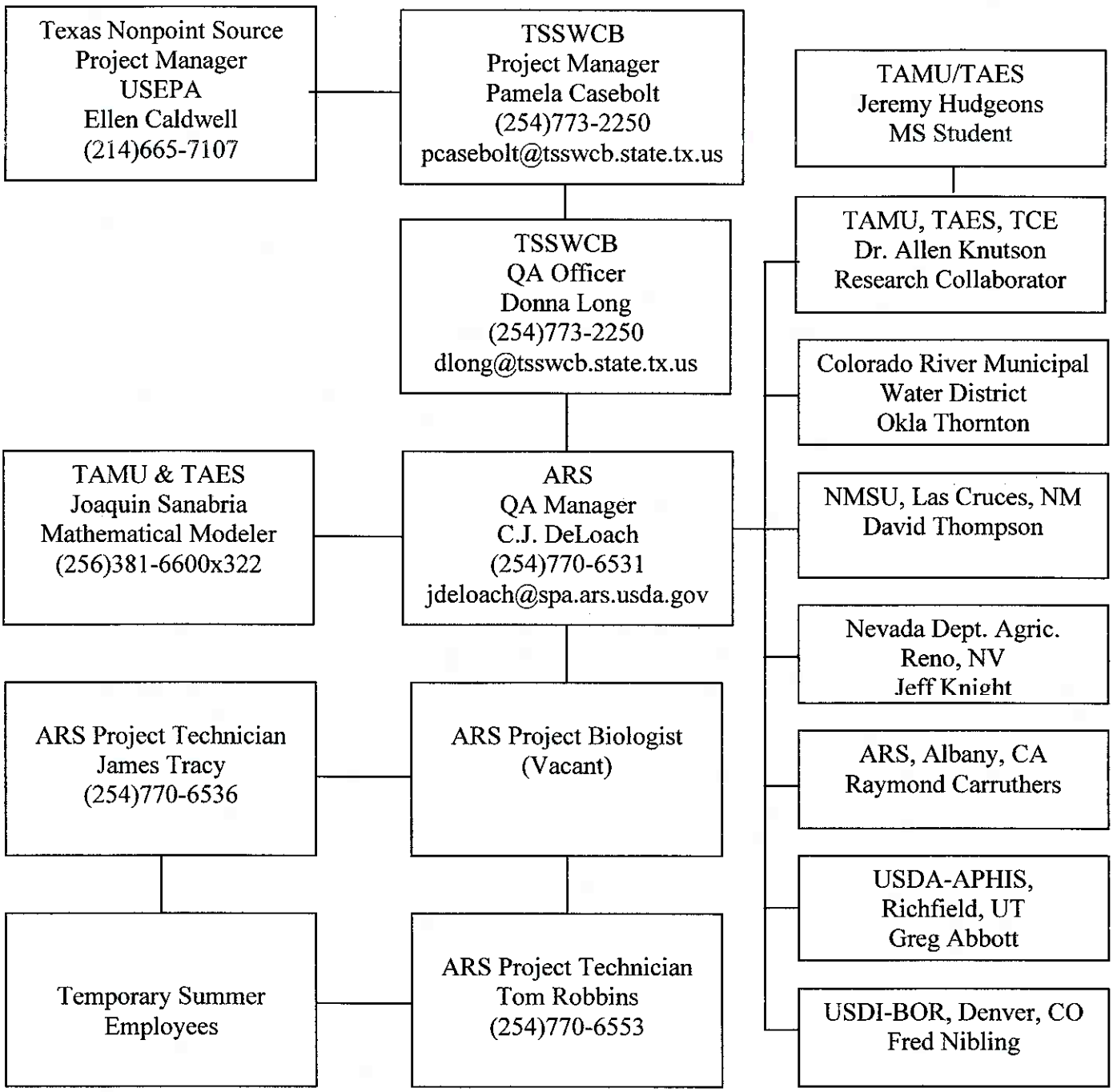
Greg Abbott, USDA-APHIS, Richfield, UT – Authorizes sampling and use of field data at the Delta, UT release sites of *Diorhabda* beetles.

David Thompson, Dept. Entomology, NMSU, Las Cruces, NM – Authorizes sampling and use of field data and aerial remote sensing imagery at the *Diorhabda* release sites near Artesia, NM.

Principal Data Users

- Ranching community (ranchers, landowners)
- USDA (ARS, APHIS, FS, NRCS)
- USDI (BR, BLM, NPS, FWS, BIA)
- USDOD (COE, Army, Air Force, Marines)
- State Agencies: Departments of Agriculture and Parks and Wildlife Departments in Texas, New Mexico, Arizona, California, Nevada, Utah, Colorado, Oklahoma, Kansas, Nebraska, South Dakota, North Dakota, Wyoming, Montana and Oregon, TSSWCB, TX Invasive Plant Species Council, Texas Noxious Weed Committee, Texas Commission on Environmental Quality
- Universities: TX Agricultural Extension Service, TX A&M University, Texas Tech University, University of California, University of Nevada, Utah State University, Colorado State University, University of Montana, other western universities, NM State University
- Water users: agricultural, residential, municipal, industrial, environmental; Colorado River Municipal Water District, other water districts
- Environmental organizations: Nature Society, Sierra Club, Audubon Society, Lower Colorado River Authority, International Boundary and Water Commission, Rio Grande Institute, Environmental Defense Fund, and others

Fig. A4-1. Project Organization Chart



Section A5: Problem Definition/Background

A. Need for control of saltcedar

1. Historical Perspective

a. Introduction, spread, area infested. The invasion of native plant communities along western U.S. streams and lakeshores by exotic saltcedars (*Tamarix* spp.), small trees or shrubs from Eurasia and Africa, has produced one of the worst ecological disasters in the recorded history of the region. The plant was first recorded in a nursery in New York in 1823, and thereafter it was widely planted throughout the West as an ornamental and to control streambank erosion. It had escaped cultivation by the 1890s, it was noted as a pest in some areas by 1910, it rapidly invaded riparian areas after the late 1920s, and by 1950 it occupied large areas of many western riparian areas (Robinson 1965). Today, it occupies ca. 2 million acres of prime riparian bottomlands and it is still spreading along tributaries and small streams. Worldwide, 54 species are recognized throughout temperate Asia, southern Europe and northern Africa, and along eastern Africa to southern Africa. Two centers of speciation developed, one from central Asia to China and in the eastern Mediterranean area (Baum 1978). Some 10 species have been introduced into the U.S. (Baum 1967, Crins 1989); 4 of them, and their hybrids (Gaskin and Schaal 2002), cause almost all of the damage (reviewed by DeLoach and Tracy 1997, DeLoach et al. 2000, 2003) (Fig. A5-1; Fig. A5-2).

b. Types of damage caused.

(1) Water usage. Saltcedar thickets typically use 4 to 5 feet of water per unit area per year, that in the present drought severely reduce water available for agricultural irrigation, and for municipal and environmental use. This is causing default of water agreements between states and between the U.S. and Mexico and damages parks and natural areas. The estimated value of the water lost to saltcedar is $133-285 million annually (Zavaleta 2000), plus other large environmental losses (Brown et al. 1989). The low flow through the Rio Grande and other streams lowers water quality by exacerbating the accumulation of pollutants in stagnant pools used by municipalities, agriculture and wildlife. Bureau of Reclamation estimates that ca. one-third of the allowable diversion of water from the Rio Grande is used by the huge thickets that occur from Albuquerque to Lake Amistad and along the two main tributaries, the Pecos River below Santa Rosa NM and in TX and along the Río Concho, Chihuahua. In several areas along the Rio Grande between Presidio and El Paso, TX and along the Pecos River from Carlsbad to Artesia, NM the sediment deposit and bank aggradation caused by dense saltcedar thickets has caused the river channel to disappear into numerous small, meandering streams spread out across the floodplain. Releases of water must be increased by 50 to 100% just to push the water through the saltcedar thickets and other exotic, invasive aquatic weeds further downstream. A large portion of the acute shortage of water needed for agriculture along the Rio Grande/Rio Bravo in both Texas/New Mexico and in Mexico is because much water is used by saltcedar. (See Appendix A for more details).

(2) Environmental: Native plant communities and wildlife habitat. Saltcedars often completely displace native riparian plant communities, degrade wildlife habitat, increase wildfire frequency and soil salinity, and interfere with recreational usage of natural areas. These invasive shrubs increase bank aggradation, narrow and deepen stream channels, alter water temperature and quality, and damage the habitat of many aquatic invertebrates, fish and riparian animals by eliminating backwaters and sand and gravel bars, and by changing riffle and bank structure. Native insects and other animals did not evolve with saltcedar and are unable to utilize it as a food resource, except that many pollinating insects (all produced on native plants) visit its flowers where they may be fed upon by some types of birds; also, wildlife and livestock may browse the foliage of young plants. Altogether, some 51 threatened or endangered (T&E) species occur in saltcedar-infested riparian ecosystems in the western United States, including 34 fishes, 6 birds, 3 mammals, 4 amphibians and reptiles, 1 insect and 3 plants. Saltcedar appears to cause minor or major damage to 48 species, to have no effect on 3 species, and to be beneficial to none (reviewed by DeLoach and Tracy 1997, DeLoach et al. 2000, Dudley et al. 2005).

(3) Increased soil salinity and wildfires. Saltcedar is able to use groundwater at much higher salinity levels than can other riparian plants, then excretes the excess salt through leaf glands that drips to the soil surface or falls to the surface with the autumn leaf fall. This produces a layer of salt on the soil that prevents the germination and growth of most other plants.

Saltcedar trees are highly flammable and they accumulate large quantities of dry leaf litter on the soil that also is highly flammable. The reduction of ground and surface water, bank aggredation, and the action of upstream dams reduce the incidence of floods that otherwise would wash out the saline litter. The high soil salinity, dense canopy cover, and often drought conditions reduces the growth of understory vegetation that otherwise would damp the fires. Fires are destructive to riparian areas because they kill cottonwoods and other valuable fire intolerant trees and shrubs, and saltcedar infested areas burn much more frequently than stands of native riparian vegetation. Saltcedars are fire tolerant and often regrow from the plant base to a height of 8-10 ft. the next year.

(4) Parks, recreation. Saltcedar acts in many ways to reduce the recreational usage of parks and natural areas. Saltcedar reduces or eliminates the attractive and cooling effects of upper canopy of cottonwoods and willows in campgrounds and recreational areas, block access to streams and shores for camping and fishing, birdwatching, hunting, etc., and reduces the aesthetic beauty of natural areas.

c. Conventional Controls. Cost of the preferred herbicidal treatment (aerial applications of Arsenal) is ca. $220/acre and mechanical and other controls may be even more expensive. Inevitably, these controls last only a few years because of resprouting and reinfestation from wind and water borne seeds (Taylor and McDaniel 1999). Aerial applications of herbicides are difficult to apply in some areas and kill native vegetation where saltcedar and the natives occur in mixed stands. In many areas where saltcedars have been removed, streams have flowed again or increased in flow, and native plants and wildlife have increased (Duncan, 1997, Eagan 1997, Barrows 1998). (See Appendix A for more complete discussions).

d. Biological Control. Biological control offers an innovative management technique that promises permanent, low-cost, environmentally safe control of large or small stands in all habitat types, in areas with difficult access, in stands of mixed native/saltcedar vegetation, and that can be integrated with other control method to extend their treatment life. Classical biological control of weeds has been used worldwide since 1865 in 75 countries against 133 weed species (Julien and Griffiths 1998). The philosophy and general protocols are well developed (Huffaker 1957, Wapshere 1974, Spafford-Jacob and Briese 2003). It has been used in the U.S. and Canada against some 40 serious exotic, invasive weeds since 1945. Biological control of 12 of these has resulted in complete or substantial control over most of the infested area. Success since the 1970s, with increased effort and better technology, approaches 70%. In the more successful cases, no other control has ever been needed (DeLoach 1991, Nechols et al. 1995, Rees et al. 1996). No case is known of eradication of a weed by biological control or by any other method of control. The objective of biological control is to permanently suppress the weed population below the threshold of damage, and without harm to non-target plants, and with low populations of both the weed and the control agent remaining.

2. Scientific Aspects.

a. Origin and taxonomy of saltcedar. Taxonomists recognize 54 species of *Tamarix* in the Old World, with a primary center of origin in central Asia and a secondary center in the eastern Mediterranean area; none are native in the Western Hemisphere (Baum, 1978). Ten species have been introduced into the United States since 1823 (Crins, 1989), and 4 species (and their hybrids) have become aggressive weeds, *T. ramosissima*, *T. chinensis*, *T. canarienisis* and *T. parviflora* (Baum, 1967). All are aggressive, cold tolerant deciduous shrubs or small trees. One other species, *T. aphylla* (athel), is a large, evergreen, cold intolerant, and not very aggressive tree used in the southern areas of the U.S. and in northern Mexico as a low-value shade tree and for windbreaks; it is not a target for biological control.

b. Natural enemies of saltcedar. In the Old World, over 300 species of mostly host specific insects, and a very few plant pathogens, are known herbivores of saltcedar (Sidnanski 1968, Gerling and Kugler 1973, Habib and Hassan 1982, Kovalev 1995). Overseas, some 20 of these species have been tested to some extent, and 10 species have been tested in quarantine in the U.S.

c. The *Diorhabda* leaf beetle. A leaf beetle, *Diorhabda elongata*, was the first beetle to be completely tested and approved for release (see Fig. A5-3, page 12a). Both larvae and adults feed on the foliage of saltcedar. The larvae pupate and the adults overwinter under litter on the soil and can drown during floods. It is the most widespread and damaging natural enemy in Asia and occurs from central China to eastern Turkey. The biology and field results obtained with these *Diorhabda* indicate that they are capable of controlling both old and young saltcedar plants in a variety of habitats, although in flood-prone areas they may drown and in some habitats populations may be reduced by predators (ants, lady beetles, assassin bugs etc.) (Lewis et al. 2003b). (See Appendix A for more details).

B. Objectives

The general objective of this proposal is to use mathematical models to demonstrate the rate of dispersal of the *Diorhabda* leaf beetles after release into the open environment, and of their defoliation of saltcedar, and to identify factors that affect the dispersal of the insect and the defoliation of saltcedar by the insect. The models will include climatic factors (air temperature, canopy temperature, RH, rainfall, wind direction and velocity, solar radiation) and biotic factors (reproductive rate and mortality of the beetles including predation, *Tamarix* foliage density), that influence the rate of dispersal and that will explain why the dispersal rate may vary under different conditions and at different locations. More specifically, the objectives of the construction, demonstration, and functioning of the models are to allow:

1. Agencies and individuals to plan how far apart releases of beetles should be made in order to obtain control within a certain area within a given period of time.
2. Better decisions in comparing the desirability, cost and rapidity of different methods of saltcedar control, such as herbicidal, mechanical or biological.
3. Wildlife managers (especially of the endangered southwestern willow flycatcher, which has begun nesting in saltcedar in some areas, especially in mid-elevational areas of Arizona), to predict when (or if) beetles released in other areas will disperse to flycatcher breeding areas. With predictions from the model, the managers can begin preparations to enhance the native stands of willows and other native plants that are the natural nesting habitat for this flycatcher. Also, it will allow wildlife managers to incorporate biological control of saltcedar into the recovery plans of other threatened and endangered species, probably at great savings in funds that would be spent for other control methods.
4. Water districts and water management agencies to predict when biological control would reduce saltcedar densities in certain areas and therefore to incorporate biological control of saltcedar into the overall water management plan, again at substantial monetary savings.
5. To compare the adequacy of purely physical (diffusion) models with a statistical model to study the interaction of *Diorhabda* with characteristics of the environment where the insect has been introduced to control *Tamarix*.

Section A6. Project/Task Description

A. Outline of Tasks

1.1 The ARS and TAES, in cooperation with TSSWCB, will develop a QAPP and submit it to EPA before data collection begins.

1.2 Develop trial models based on other types of diffusion and dispersal models from the literature.

1.3 Establish field plots for monitoring beetle dispersal and defoliation of saltcedar at sites near Big Spring in northwestern Texas.

1.4 Conduct periodic monitoring that will include the abiotic factors (temperature, relative humidity, rainfall, wind speed and direction and solar radiation) and biotic factors (saltcedar canopy density, plant vigor, degree of defoliation, beetle reproductive rate and mortality, predation, and defoliation by competing herbivores) that are likely to affect the rate and intensity of dispersal. Monitoring will be conducted weekly or at the peak of each beetle generation by ARS and TAES personnel and by their trained field technicians.

1.5 ARS and TAES will prepare quarterly progress reports and a final report on the data collection and on the model development and validation to TSSWCB.

B. Background Information on the Model

Dispersion of many insects in nature resembles the physical phenomenon of diffusion. This can be represented mathematically by partial differential equations that describe changes of insect population with respect to space and time variables. A key element in those equations is a term equivalent to the diffusion coefficient. Kovalev and Vechernin (1986) developed and applied an Isolated Population Wave (IPW) model that represents a wave movement similar to that followed by fire in a prairie; they used the model to study the dispersion of the ragweed beetle *Zygogramma suturalis* F. which is used to control ragweed *Ambrosia artemisiifolia* L. in Russia. A similar model initially developed by Okudo (1980) was used by Smith, et.al (2001) in forests to study the dispersal of *Anoplophora glabripennis* (Cerambycidae) which is a pest of many hardwood trees like maple and poplar. This second model was designed to be used with marked and recaptured insects but can be adapted to work with the whole insect population if the disappearance coefficient δ is changed to an appearance-disappearance coefficient; such a coefficient can be estimated mathematically in the way Smith, et. al. (2001) did and is explained later. The mathematical calculation of the appearance-disappearance coefficient δ can be validated experimentally with experiments in field cages. Another parameter required to adapt the model to work with the entire insect population is the original beetle population p_0 at the beginning of the growing season.

C. Existing Data to Support the Model

The *Diorhabda* dispersal model will draw heavily on the 4 years of dispersal data available at Lovelock and Schurz, NV, Delta, UT, Pueblo, CO, and Lovell, WY. The releases of *Diorhabda*

beetles made during August 2003 at Seymour, Lake Thomas, Big Spring, TX and Artesia, NM will provide data on dispersal, beginning with the date of release. This gives a total of 6 years ofdata at the 5 northern sites and 3 years of data at the 4 southern sites by the end of the grant period. Monitoring at the Texas sites can be adjusted to meet the needs of the model. This work is funded in part by EPA grant C9-996236-11 through the Texas State Soil and Water Conservation Board (Grant # 03-11).

D. Development of the Model

One limitation of the physical models like those described above is the lack of biological explanation for the diffusion coeffecient and other parameters, in other words, the physical models are able to describe the dispersal of insects but do not tell us why they disperse or what are the biotic or abiotic factors that move the insect population to colonize an area. The two physical models will be used together with a Spatial Multiple Regression Model (SMRM) (Schabenberger and Pierce 2002). The diffusion models will estimate the rate of dispersion of *Diorhabda elongata* and the SMRM will identify biotic and abiotic factors that affect the dispersion of the beetle and the defoliation of saltcedar by the beetle. In a SMRM for the prediction of saltcedar defoliation, the initial *Diorhabda* population at the beginning of a growing season should be one of the most important predictor variables in the model. The same basic data of beetle larvae and adults counts at sample points in the transects, made every week at the beginning of the growing season and at the peak of the generation later in the season is needed for the two physical models. The three models will be applied to adults and larvae of *Diorhabda*, and a correlation analysis of model predictions with saltcedar defoliation will identify which stage of the insect is better for modeling insect dispersion. Additional variables that will be measured at transect points for the statistical model will be saltcedar biomass, ground cover area by saltcedar, saltcedar foliage vigor, canopy and air temperature, relative humidity and radiation interception by the saltcedar canopy.

1. Okudo's Diffusion Model

The diffusion model used by Smith, et. al. (2001) was originally developed by Okudo (1980), it has been tested in studies by Shigeda and Kawasaki (1997) and by Turchin (1997). It is based in the following differential equation:

$$\frac{\partial n}{\partial t} = D\left(\frac{\partial^2 n}{\partial x^2} + \frac{\partial^2 n}{\partial y^2}\right) = D\left(\frac{\partial^2 n}{\partial r^2} + \frac{1\partial n}{r\partial r}\right) \qquad [1]$$

Where n is the number of beetles per unit of area or tree, t is time in weeks, D is the diffusion coefficient, x and y are spatial coordinates. The spatial coordinates can be transformed to radial distance using the expression $r = \sqrt{x^2 + y^2}$

The diffusion coefficient determines the rate at which the beetles move, which may change with the direction of movement, the distribution of saltcedar trees, obstacles in the path of movement, and environmental variables like wind direction and speed, and orientation to the sun angle.

The dispersal of the saltcedar beetle will be studied using the solution of equation 1:

$$n(r,t) = \frac{\delta p_0}{4\pi Dt} \exp\left(-\frac{r^2}{4Dt}\right) \qquad [2]$$

Where n(r,t) is the number of insects at a distance r in meters at time t in weeks, δ is a coefficient for the appearance-disappearance of beetles, p_0 is the original number of beetles at release time, or an estimate of the beetle population at the beginning of a growing season.

The diffusion and appearance-disappearance coefficients can be estimated using the distribution of beetle abundance through several distances from the release point during a number of weeks. Equation 2 is fitted, $nf(r^2)$, using least squares for each week. Smith, et. al. (2001) counted the number of insects at nine distances in each of eight weeks. Another way to estimate the diffusion coefficient and the advance distance were suggested by Karaeiva (1983) using the following :

$$D = \frac{r_a^{\,2}}{\pi t} \qquad [3]$$

$$r_{.98} = 2\sqrt{4Dt} \qquad [4]$$

where r_a is the average distance of displacement of the beetles for a week, and $r_{.98}$ is the radius reached by 98% of the original beetle population.

Rearranging equation 3 the average distance of displacement by the beetles can be estimated for each week:

$$r_a = \sqrt{\pi Dt} \qquad [5]$$

Smith, et.al. (2001) also used another approach to estimate number of insects at a given distance using the following equation:

$$n(r) = \frac{p_0 (8\pi)^{-1/2} (\delta D^3)^{-1/4}}{r^{1/2}} \exp\left(-\frac{r}{(D/\delta)^{1/2}}\right), \qquad [6]$$

where *n(r)* is the total abundance across time at a distance. This equation depends only on distance, and the diffusion and appearance-disappearance coefficients can be estimated from the distribution of abundance at a number of distances; Smith, et. al. (2001) used nine distances.

2. Kovalev's Isolated Population Wave (IPW) Model

Kovalev and Vechernin (1986) developed an IPW model to study the spread of the ragweed beetle *Zygogramma suturalis* F. (Chrysomelidae) during the control of ragweed *Ambrosia artemisiifolia* L. in the field infested by this weed in Russia. The model is formulated as a diffusion equation as follows:

$$\frac{\partial n}{\partial t} = -\nabla I + f(\mathrm{n}), \qquad [7]$$

where n(r,t) is the insect density, number of insects per m^2 at a given place r at a particular time t; I (r,t) is the vector of insect flux; f(n) is the insect birth rate minus insect death rate per unit time per unit area; and ∇ = gradient = ∂/∂r .

Equation 7 indicates that the change in the number of insects at a given place is equal to the difference between the insects that have migrated to a particular point and the number of insects that have left the same point including the difference in the number of births and deaths at the same place.

The vector I of insect flux is equal to:

$$I = -D\nabla n + B\nabla p, \qquad [8]$$

where D is the coefficient of diffusion which is proportional to the gradient of insect density and describes the movement of insects from high to low density. The second term shows B, the coefficient of food search efficiency proportional to the gradient of plant density, the insects move from places of low plant density to places of high density. Plant density at a given place r and a time t is designated as p(r,t).

The change of plant material available under the influence of insect feeding is given by the equation:

$$\frac{\partial p}{\partial t} = -An, \qquad [9]$$

where A is the amount of biomass eaten by one insect per unit time. The amount of biomass eaten by the insects in a day in an area unit is equal to the biomass eaten by an insect multiplied by the number of insects in that area unit.
Assuming that the coefficient of diffusion is constant, and replacing equation 8 in 7, the IPW model for the dispersal of the saltcedar beetle can be expressed by the following system of differential equations:

$$\begin{cases} \dfrac{\partial n}{\partial t} = D\Delta n - \nabla(B\nabla p) + f(\mathrm{n}) \\ \dfrac{\partial p}{\partial t} = -An, \end{cases} \qquad [10]$$

where $\Delta = \dfrac{\partial^2}{\partial x^2} + \dfrac{\partial^2}{\partial y^2}$ is the Laplace operator.

The solution of the equations in 10 yields the speed of the wave in equation [11], the shape of the IPW wave in equation [12], the damage caused to saltcedar by the beetle population in equation

[14], and the width of the insect wave in equation [15].

$$V = \sqrt{A.B} \qquad [11]$$

The speed of the wave depends only on A, the amount of biomass eaten by an individual insect in a day, and B a coefficient of food search efficiency.

$$n(x,t) = \frac{3n_0}{2\cosh^2\left[\dfrac{1}{2}\sqrt{\dfrac{E}{D}}(x - x_0 - V_t)\right]}, \qquad [12]$$

where n_0 is a critical insect density at which the birth rate is equal to the death rate, it is the point where the following quadratic polynomial intercepts the n axis. Also, one characteristic of the IPW is that the maximum insect density, at the top of the wave, is approximately equal to $3n_0/2$

$$f(n) = -En + \frac{E}{n_0}n^2 \qquad [13]$$

The coefficient E is the slope of the linear component in equation 13, $x - x_0$ is the distance between the initial position of the wave x_0 and any point in the path of the wave, V_t is the speed of the wave at time t, and cosh is the hyperbolic cosine.

The damage caused to saltcedar by the beetles feeding on it is given by

$$P(x,t)\% = \frac{1}{2}\left\{1 - \tanh\left[\frac{1}{2}\sqrt{\frac{E}{D}}(x - x_0 - Vt\right]\right\}.100, \qquad [14]$$

where tanh is the hyperbolic tangent.

The width of the wave is represented by equation 15

$$L = 4\sqrt{\frac{D}{E}}\ln(\sqrt{2}+1), \qquad [15]$$

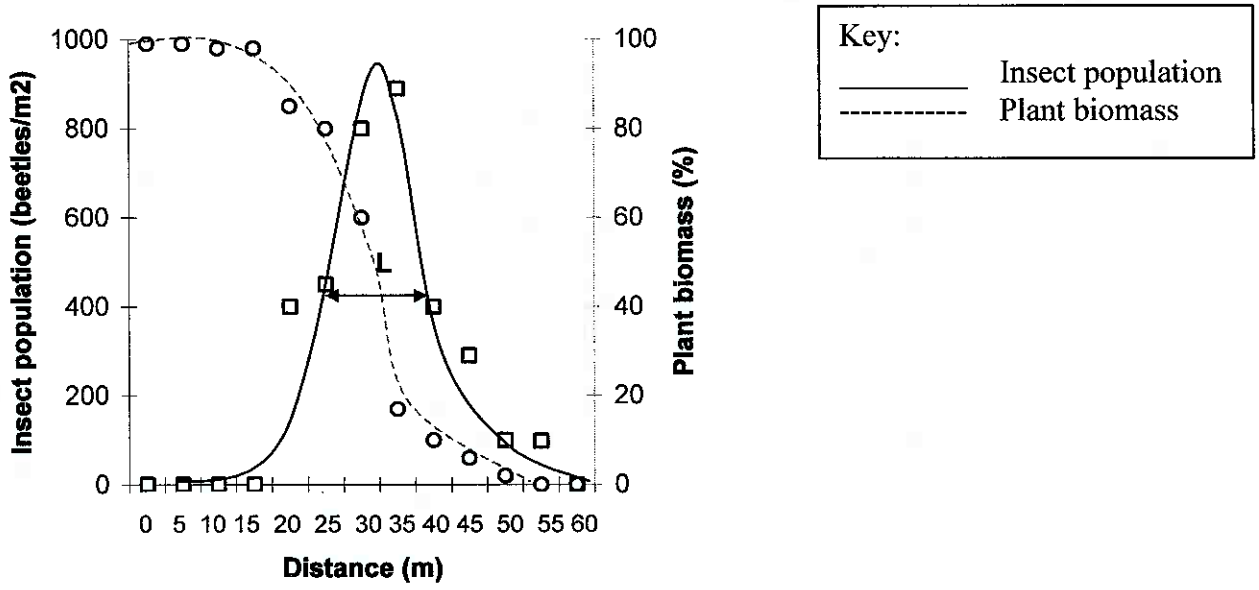


Figure A6-1. Insect population and plant biomass from field experimental data and IPW model application. (Adapted from Kovalev and Vechernin (1986) in a study of *Zygogramma suturalis* dispersal to control ragweed in Russia.)

Figure A6-1 shows the experimental data collected and curve calculation of Kovalev and Vechernin (1986) for July 9th, 1985, for the beetle population (squares) and plant biomass (circles), and the curves calculated from equations 12 and 14 for the waves of beetles (continuous line) and plant biomass (dotted line) respectively.

Kovalev and Vechernin (1986) used this figure to estimate L from the experimental data, then using equation 15 the ratio E/D can be calculated to be used in equations 12 and 14. The other parameter *Vt* in equations 12 and 14 can be estimated by plotting several weekly periods and calculating the average distance between peaks of beetle population.

3. Spatial Multiple Regression Model

The general form of a SMRM is described by Schabenberger and Pierce (2002) as follows:

$$Z_1(s) = \beta_0 + \beta_1 Z_2(s) + \beta_2 Z_3(s) + \ldots + \delta(s) \qquad [16]$$

Where $Z_1(s)$ is the prediction of *Diorhabda* population or saltcedar defoliation at point s; β_0, β_1 and β_2 are the parameters of the model; $Z_2(s)$, $Z_3(s)$.... are biotic and abiotic variables that explain $Z_1(s)$; and $\delta(s)$ is the error term.

$\delta(s)$ in equation 16 accounts for the spatial structure of the area where *Diorhabda* population or saltcedar defoliation prediction will be done. Such spatial variability structure is estimated with variograms, and covariograms.

The SMRM will be used for the double purpose of identifying critical variables that contribute to the explanation of *Diorhabda* dispersal, and to predict beetle populations and degrees of saltcedar defoliation in areas where the insect is being introduced. The SMRM can also be incorporated to a Geographical Information System to make predictions and to build maps in large areas colonized by *Diorhabda.*

E. Model Parametrization

The field data collected during the growing seasons will be used to calculate the parameters required by the models, to identify the factors that influence beetle dispersal, to determine which of the trial models provide the most accurate and most efficient prediction of dispersal, and to modify and adjust the models to obtain better prediction capability.

1. Okudo's Diffusion Model

Parameters δ and D of equation 2 in Okudo's model will be estimated through fitting the exponential model (equation 2) of beetle number as a function of distance. Regression coefficients will be estimated by least squares. The regression coefficient associated with the radial distance squared (r^2) will yield the diffusion parameter D, then it is used in the independent

term of equation 2 to obtain δ the coefficient of appearance-disappearance. Estimation of parameters δ and D will have lower uncertainty if the regression models needed are fit for at least five weeks, averages of those weekly estimations will be used as δ and D in the calculation of beetle numbers with equation 2.

Validation of the appearance-disappearance coefficient δ will be done with field experiments in cages and in the open field, employing the concept of Intrinsic Rate of Natural increase of an Insect Population (Birch, 1948). Conduction of the experiments and evaluations of the *Diorhabda* populations will follow guidelines established by Lewis, et. al. (2003b) in the study of the biology of *Diorhabda elongata.*

The parameter p_0 in equation 2, is the initial number of beetles released in the first season, or the starting population at the beginning of the second season and later seasons. A sampling of beetles will be done early in the season to estimate the population of adult *Diorhabda* beetles emerging from winterization; that estimate will be p_0 for the new season.

2. Kovalev's IPW Model

Parameters n_0, E/D, Vt, and x_0 will be estimated from a set of consecutive beetle population waves. Each weekly wave will have the form shown in Figure 1. The width of the wave L will be obtained in the form indicated by Figure A6-1, then the L value will be used in equation 15 to obtain the ratio E/D. Average distance between wave peaks will be used to calculate Vt at different periods during the growing season. The value of x_0 is the distance from the release site, or from the front advance line of the last growing season, to the point where the first insect wave appears, that point will also be identified from graphing consecutive weeks. To estimate parameter n_0 which is the population size at which birth and mortality rates are equal, we will use the property $(3\ n_0/2)$ = Maximum insect density. Maximum insect density is taken from the experimental waves at different periods during the growing season.

3. Spatial Multiple Regression Model

The β_i parameters of the SMRM will be estimated with the conventional methodologies of least squares or maximum likelihood. Only those parameters that show significance at a significance level of 0.1 will be kept in the model. The spatial structure of the area where *Diorhabda* is dispersing is described by the covariance function **δ(s)** which is the error term in the model. The function describes the degree of variability between pairs of points along the transects and how variability increases as points get separated by larger distances. The covariance function most of the time is described by exponential, spherical, or gaussian models. These non-linear models will be tried iteratively to come out with the best model to be used in the error term of the SMRM.

SAS (Statistical Analysis System) procedures such as Proc Mixed, Proc Nlin, and Proc Variogram (SAS Institute Inc., 1990) will be used for the fitting and assessment of the SMRM.

F. Acquisition of Data for the Model

This proposal for development and demonstrations of a *Diorhabda* dispersal model is closely related to the ongoing project under the Clean Water Act Section 319(h), "Upper Colorado Saltcedar Control Project: Biological Control Component" of the Texas State Soil and Water Conservation Board Grant #03-11, and EPA Grant #C9-996236-10. Some of the data to be collected under that grant will be used in the development and validation of the model.

1. Existing Data.

Some of the data from the ongoing project, "Upper Colorado Saltcedar Control Project: Biological Control Component", under the Clean Water Act Section 319(h) of the Texas State Soil and Water Board Grant #03-11, and EPA Grant #C9-996236-10 will be used in the development of the model. Also, agreements have been obtained for using data from releases made in May 2001 at Lovelock and Schurz, NV, Delta, UT and Pueblo, CO (sites with Fukang/Chilik beetles well established) and at Lake Meridith in northern Texas. Thus, data is available from sites where releases are planned during 2005, and from sites where releases were made last year or 5 years ago. This data from where dispersal has already been in progress is valuable in constructing the model, since it is equivalent to observing one site for 5 years (see Section B1-A).

2. New Data.

The major beetle release site that will contribute data to the model is located along Beals Creek, ca. 3 mi east of Big Spring, TX (Higgins Ranch). The Crete beetles were first released there in April 2004, and additional ones during July and August and were recovered in April 2005; this and Lake Meredith are the only locations in Texas where the beetles are established in the open field. Other possible locations are along Sulphur Springs Draw and at the CRMWD reservoir ca. 10 miles west of Big Spring, at Lake Thomas (Site a & b) ca. 35 miles northwest of Big Spring, at Seymour in north central TX, and at Ft. Stockton, TX. Another site is at Artesia, NM where the beetles seem to be established. (See Section B1-B.1 for a description of the releases and maps of the sites).

3. Data for Various Parameters. Collection of data for the various parameters of the model are discussed in Section B2.

G. List of Products, Deliverables, Milestones, Schedules

1. Year by Year Activities

   a. Year 1

(1) Literature review and preliminary model. Conduct a review of the literature to determine what types of mathematical models have been developed in the past that describe the dispersal of insect populations in nature and the factors that may affect dispersal. Adapt these models to the dispersal of the *Diorhabda* leaf beetles and construct a trial model to describe the dispersal of these beetles when released in saltcedar stands for biological control.

(2) Establish field experimental plots. Establish an experimental area in a saltcedar stand in the open field near Big Spring, TX where the *Diorhabda* beetles were released in 2004. The plot will consist of sampling quadrats located along transects across a saltcedar stand and along Beals Creek that can be extended several miles upstream and downstream. Install weather monitoring instruments at the plot and among the saltcedar plants.

An additional site will be established in the Big Spring, TX area if beetles released in 2004 have established, where less intensive monitoring will be conducted as a replicate for comparison with the Beals Creek site.

(3) Monitor beetle dispersal and influencing factors. In the low-density stand of saltcedar at Beals Creek, monitor *Diorhabda* beetle dispersal from the original release point to measure the velocity, width and direction of the advancing wave of beetle dispersal and saltcedar defoliation through weekly (at first) and later monitoring at the peak of 3rd instar larval populations of each of the 4 to 6 annual generations. Also, we will record weather data (temperature, RH, rainfall, wind direction and velocity, and solar radiation) and biotic variables (beetle populations, saltcedar density, condition, and degree of defoliation, predation of the beetles, and damage to saltcedar from another insect herbivores.

At Lovelock, NV, monitor similar factors in the large, dense saltcedar stand where the beetles now have defoliated some 5,000 acres of saltcedar and have migrated ca. 100 miles along the Humboldt River. A reconstruction of the generational dispersal waves of defoliation during the past 4 years can be obtained here from remote sensing photography. Monitoring will be done twice annually, as only two generations a year are produced there.

This monitoring will be performed by ground-level counts of beetles and evaluation of saltcedar foliage, by the use of pheromone traps to sample low populations of beetles and by low-level aerial photography.

b. Years 2 and 3

The dispersal rate of the released *Diorhabda* beetles, based on the previous 4-year experience at Lovelock, NV, Schurz, NV, Delta, UT and Pueblo, CO, is expected to increase geometrically during at least the first several years after release. At Lovelock, the beetles had dispersed over a radius of ca. 100 m after the second year (2 acres defoliated), 500 m after the third year (500 acres defoliated), and 160 km along the river after the fourth year (5,000 acres of a continuous dense saltcedar stand defoliated).

During years 2 and 3, monitoring will continue as in the first year, but the transects will be extended during each year to stay ahead of the advancing wave of beetle dispersal. Both the velocity and the width of the advancing wave are expected to increase. The various abiotic and biotic factors will be evaluated and those having only minor influence on the rate of dispersal may be dropped. Improvements in the accuracy and sensitivity of the model and its ability to describe the increasing rate of dispersal will be made after each years data is collected. At least 3 years of monitoring data are required to establish the increasing rate of dispersal. Remote sensing data will provide a measure of the rate of defoliation at the Beals Creek and other release sites that may be established. By the end of the third year, a total of 7 years of dispersal data will be available from the Lovelock and other northern sites that will be incorporated into the model.

Section A7: Data Quality Objectives and Criteria for Measurement Data

Objectives of the biological monitoring are that the data will be accurate, representative, comparable with other sites and programs on biological control of saltcedar, and complete. The project biologists and technicians will collect data on populations of *Diorhabda* beetles and their predators, other insects that damage saltcedar, saltcedar canopy cover, defoliation, foliage condition, biomass and light interception by the trees, and remote sensing will provide a standard for measuring defoliation. The several methods used (visual estimates, photographs of foliage on branches, biomass and remote sensing) will be used to calibrate the accuracy of the others. A variety of weather variables will be monitored, including temperature and relative humidity within the foliage canopy at different levels; and at a control weather station, rainfall, wind direction and velocity and solar radiation. Some or all these biotic and abiotic variables are expected to influence beetle dispersal.

A. Estimated Accuracy of Field Monitoring

To assure accuracy, the counts made by the field technicians will be compared immediately afterwards to counts on the same branch made by the experienced ARS technicians until the field technician counts are within 10% of the counts made by the experienced ARS personnel at the same time and location. Relative percent difference (RPD) will be calculated by the formula:

$$\frac{\text{field technician}}{\text{ARS technician}} \text{X } 100.$$

This procedure will be repeated weekly until the field technician counts are consistently within 10% RPD. Variance of the field monitoring data will be tested by statistical analysis of the data at 80%, 90% and 95% confidence limits.

Accuracy of the field identification of plants and insects by the ARS technicians (Robbins and Tracy) will be determined by comparison with insects and plants from the same collections (location and date) made by taxonomic specialists at SEL, NMSU, TX A&M, or the University of Texas, Austin.

B. Representativeness

The sites selected near Big Spring are representative of western Texas riparian habitats and include mixed native/saltcedar and monotypic saltcedar vegetation community types, reservoir floodplains, and streamsides, upland areas and reservoir shorelines. The sites at Lovelock, NV and Pueblo, CO where more limited comparative monitoring will be done are representative of much of the saltcedar infestation at those latitudes.

C. Comparability

The sampling conducted here is more detailed than that at other sites in Texas and to ongoing sampling in 7 other western states, but comparisons between areas still can be made. Origin of the *Diorhabda* released in each location as well as differences between *Tamarix* genotypes and climatic variables at each location are important factors considered for the comparisons of *Diorhabda* dispersal in the different sites.

D. Completeness

Weather conditions may prevent collection of some samples; in each case, documentation/field notes of such adverse conditions will be recorded.

Any changes to the monitoring sites listed will be made as an amendment to the QAPP.

Although 100 percent of collected data should be available, accidents, insufficient sample volume, or other problems must be expected. A goal of 90 percent data completeness will be required for data usage. Should less than 90 percent data completeness occur, the Program Manager will initiate corrective action procedure (Quality Control Requirements Section B5).

Data completeness will be calculated as a percent value and evaluated with the following formula:

$$\% \text{ completeness} = \frac{SV}{ST} \times 100$$

where: SV = number of valid samples collected
ST = total number of samples scheduled for collection

Database checks for validity will be performed on an on-going basis. Data will be reviewed for abnormalities or any unusual results, prior to entry into the database. Any unusual results will be traced for error sources. In the event no error is found, the data will be assumed normal and appropriate for decision determinations. If an error is found and cannot be resolved, that datum will be discarded.

The Project Manager will coordinate with Field Biologists and technicians to ensure that proper protocols are being utilized.

E. Model Assessment

Uncertainty of physical deterministic models like the two used in this study depends basically in the identification of the most influential parameters and in the quality of the estimation of those parameters. It is very common that individual parameters have different impacts in the variability of the model output, also it is expected that the parameters in the model do not act

independently, in most cases they interact affecting the model output variability. Literature is abundant in methods for assessing model sensitivity and uncertainty. In this study we will use the concept of total sensitivity index suggested by Ratto, et. al. (2001), and the non parametric Kolmogorov-Smirnov goodness of fit test (Conover, 1980) to asses the uncertainty of the model.

1. Sensitivity Analysis

The purpose of sensitivity analysis is to evaluate the impact that each model parameter has on the variability of the model output. It allows ranking of the model parameters in order of importance. The higher the variability in the model output as a result of changing values of a parameter, the more important the parameter is. The importance of a parameter is measured by the Total Sensitivity Index S_{Ti} (Ratto, et. al., 2001) which is the ratio of a marginal variance of Y model predictions over the total variance of predictions as is shown in the following equation:

$$S_{Ti} = \frac{E[V(Y \mid X_{-i} = x_{-i})]}{V(Y)} \qquad [18]$$

where V(Y) is the total variance of model predictions calculated from a set of Y predictions. The set of Y predictions contains subsets of Y predictions, each subset of Y predictions results from changing the values of parameter X_{-i} while keeping the other parameters at fixed values.

$V(Y| X_{-i}=x_{-i})$ is the variance of each subset of Y predictions. $E[V(Y| X_{-i}=x_{-i})]$ is the average of all the variances from all subsets. The Total Sensitivity Index accounts for the effect of parameter interactions over the model prediction variability. S_{Ti} takes values between 0 and 1, the higher the value the more influential the parameter is. Influential parameters need to be estimated with the highest precision possible since they are responsible for determining most of the model uncertainty.

Calculation of the Total Sensitivity Index involves many iterations using several values of the parameter being evaluated, and several arrangements of fixed values for the other parameters in the model, and then repeating the procedure for each of the model parameters. We will use the software GLUEWIN developed by Ratto and Santelli (2001) for the specific task of calculating sensitivity indices.

Identification of the most influential parameters in the physical models is also useful to connect those parameters to biotic or abiotic variables, for that purpose values of the parameters will be correlated with biotic and abiotic variables measured in the study. The magnitude of those correlations are expected to be characteristic of different regions and seasons.

2. Uncertainty Analysis

After selecting a set of parameter values for each model, the uncertainty of the two physical models will be assessed with the Kolmogorov-Smirnov goodness of fit test (Conover, 1980). A

1- α confidence interval for the cumulative distribution of the observed data will be constructed using 1-α quantiles of the Kolmogorov-Smirnov test statistic; α is the significance level adopted that will define the width of the confidence interval. Uncertainty of each physical model will be estimated based on the percentage of the model predicted data that falls outside the confidence interval for the observed distribution.

3. Calibration

The set of parameter values that make the model to have an acceptable level of uncertainty are not universal, they are expected to change with location and growing season. A set of parameter intervals will be estimated and tried on the model to calibrate the model to the specific conditions of location and season.

The assessment of the SMRM will be done employing statistical conventional methods such as examination of determination coefficients, tests to avoid multicollinearity, mean square of the error, and significance of each parameter. Uncertainty of the SMRM will be estimated the same way as the physical models, with the Kolmogorov-Smirnov goodness of fit test.

F. Composited Samples

Most of the sampling performed will be composite given the nature of the sample unit, which is a quadrat in a transect. Inside each quadrat are 9 randomly selected branches (3 from the top of the canopy, 3 from the middle of the canopy and 3 from the bottom of the canopy) that play the role of sub-sampling units for several of the evaluated variables like insect populations and degree of defoliation. Another variable that will be measured using subsamples from inside the quadrats will be the light interception by the canopy. In this last case the sub sampling units will be light interception readings from five locations inside each quadrat. The Project QA Manager will assure that personnel are proficient at making these measurements. If problems occur in the field sampling process, responsibility for corrective actions will be in the following order: ARS Project Manager/QA Manager (DeLoach), TAMU Cooperator (Knutson), and ARS Lead Technician (Tracy).

If a site is destroyed (as by accidental herbicide or insecticide applications or by fire) the sampling being conducted then will be moved to one of the other sites at Lake Thomas sites A or B or Big Spring site C (Buzzard Draw). If a site is temporarily destroyed by flooding, sampling will resume as the flood waters recede, as this is a part of the natural environmental system. If the collected data sheets are lost or destroyed, replacement monitoring will be conducted as soon as possible after the loss.

Section A8: Special Training Requirements/Certification

All personnel involved in sampling, sample analyses, and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. ARS and TAES personnel involved in use of global positioning system (GPS) instruments have been trained in their appropriate use.

Field personnel will receive hands-on training in insect and plant sampling by working directly with ARS, TAES and water district personnel prior to sampling/assessment activities. Certifications are not required.

The two ARS technicians assigned to the project (Robbins and Tracy) are highly skilled insect taxonomists and botanists already with 3 years experience in identifying the insects on saltcedar and on native vegetation occurring in the sample areas. During the beetle and vegetation surveys any part-time or less skilled helpers will work alongside Robbins or Tracy.

All part-time employees will work closely in the field with the skilled ARS or TAES identifiers until they become proficient in identifying the plants and insects required for monitoring.

Dr. Sanabria will visit the laboratory of Dr. Peter McEvoy, Oregon State University, Corvallis to coordinate modeling methodologies between this project and research on modeling dispersal on biological control agents at that institution.

Section A9: Documentation and Records

The ARS Quality Assurance Manager will personally distribute a copy of the most recent version of the QAPP to the project staff and discuss it with the project staff. When an updated version is produced, he will email it as a PDF file to the project staff. He will discuss changes with the project staff to ensure understanding.

Hard copies of all field and laboratory data sheets, GPS and GIS data, digital photographs, instrument calibration, corrective action reports (CARs), etc. and billing receipts will be recorded on appropriate forms (examples in Appendix B.B) and archived by ARS at the Grassland, Soil and Water Research Laboratory (GSWRL), Temple, TX for at least five years. These hard copies will be filed in folders after each field or laboratory data collection, fastened securely in place, and with tabs identifying each type of data. The data forms (see SOP Appendix B) will identify where the experiment was done, the names of the persons taking the data, and the conditions at the time of collection, and the general methodology of data collection. Data will be backed up on CD at the end of each day of data entry, the CDs will be identified and stored in a different room from where the hard copies are stored. In addition, ARS will archive electronic forms of all project data for at least five years. Hard copies of billing receipts will be kept on file by the Administrative Officer, GSWRL, Temple.

The ARS Project Manager will produce an annual quality assurance/quality control report, which will be kept on file at GSWRL with copies made available upon request. Any items or areas identified as potential problems and any variations or supplements to QAPP procedures noted in the laboratory quality assurance/quality control report will be made known to pertinent project personnel and included in an update or amendment to the QAPP.

Quarterly progress reports will note activities conducted in connection with this project, items or areas identified as potential problems, and any variations or supplements to the QAPP. CARs will be utilized when necessary and will be maintained in an accessible location for reference at ARS. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP.

Section B1: Sampling Process Design: Experimental Design

Knowledge acquired from previous field data collection during 2001-2004 at well established sites north of the 38th parallel, at Lovelock and Schurz, NV, Delta, UT, and at Pueblo, CO will be used for the sampling and experimental design at the new sites where data will be collected for the development of the dispersal models previously described. The new locations are recently established, ongoing, sites near Big Spring and other sites in Texas and at Artesia, NM if they become established. The major site is along Beals Creek, on the Higgins Ranch ca. 5 mi east of Big Spring, where the *Diorhabda* beetles were first established in April 2004 (released) and April 2005 (overwintered). Back-up sites are located along Sulfur Draw, ca. 10 mi NW of Big Spring where the beetles were released in July 2005. During the 2005 growing season field data was collected only from the Big Spring site. Data from other Texas and New Mexico locations will be used if there is progress in *Diorhabda* establishment during the 2006 and 2007 growing seasons.

B. Field Sampling to Obtain New Data.

1. Location and description of new sites.

The major beetle release site that will contribute data to the model is located in the Upper and Middle watersheds of the Colorado River Municipal Water District (Fig. B1-1), centered on the David Higgins Ranch. Secondary, or backup, sites are located along Buzzards Draw, Sulphur Springs Draw, and the CRMWD reservoir north of Natural Dam Lake, west of Big Spring, and at the upper end of Lake Thomas (ca. 35 mi northeast of Big Spring). Other sites are located on the Allsup Ranch ca. 110 miles to the northeast near Seymour, TX; along the Pecos River 14 mi north of Artesia, NM; and along Comanche Creek near Ft. Stockton, TX; as described below. Beetles from all these sites will be included in the dispersal model as needed when they become established. Criteria for selection of release sites are given in the SOP, Appendix B.1.

Big Spring – Higgins Ranch. This main site is centered on the David Higgins Ranch, and extends ca. 3 miles eastward to Moss Lake (where the herbicide treatments stopped) and westward ca. 3 miles through the sewer treatment plant to Loop 700 ca. 5 miles east of Big Spring, TX. The release site is located on the western edge of a 22 acre patch of saltcedar that extends ca. 300 m to Beals Creek (Fig. B1-2). The Crete beetles were first released there in April 2004, and additional ones during July and August. Overwintered adult beetles were found during April 2005, apparently without excessive overwintering mortality, indicating a good probability of establishment. This is the only location in Texas or New Mexico where the beetles probably are established in the open field. Saltcedar occurs almost continuously from the release point along Beals Creek for several miles both upstream and downstream. Beetles were released here in April 2004.

Big Spring – Sulphur Spring Draw and CRMWD Reservoir. Beetles were released here in April 2005, but establishment is not yet confirmed (Fig. B1-3).

Lake Thomas – Lakebed. Beetles were released here in August 2003 (Fig. B1-4, site 1a), overwintered, and reproduced well in the spring of 2004 but then the site was lost, probably to native insect predators. Beetles from the Murphy Ranch site will be re-released here as part of the site suitability study.

Lake Thomas – Murphy Ranch. In the spring of 2005, colonies of beetles were established in 20 field cages on the Murphy Ranch on upper end of Lake Thomas Reservoir (Fig. B1-4, site 1b), for a study on the effects of defoliation on carbohydrate reserves and death of the saltcedar plants. These beetles will be released into the open field when the experiment is completed in 2006.

Seymour. This site is located on the Allsup Ranch on the upper floodplain of Lake Kemp, along the Wichita River (Fig. B1-5), 13 miles NW of Seymour, TX. Beetles were released here in August 2003 and again in July 2005 but have not yet established. Beetles will be re-released here in 2006 as part of the site suitability study.

Ft. Stockton. Colonies of Crete beetles were released in field cages along Comanche Creek here in 2004 and are awaiting APHIS permits for release.

Artesia, NM. Crete beetles were released here by cooperators at New Mexico State University in August 2003 and appeared to be established but then the population was lost during the summer of 2004, apparently from attack by native insect predators. Beetles were released at a nearby site and at another site in regrowth following herbicidal control during 2005.

Carlsbad, NM. *Diorhabda* beetles from Posidi, Greece were released here in August 2003 but have failed to establish. This site is managed by the Bureau of Reclamation, Denver, CO.

Lake Meredith. *Diorhabda* beetles from Posidi, Greece were released here in 2004 and now have overwintered and now appear to be established. This site is managed jointly by Bureau of Reclamation, Denver and Texas A&M, Bushland, TX.

2. Landowner/land manager agreements. Landowner/manager agreements will be recorded (Form C-23) and kept on file at GSWRL, Temple, TX.

3. Sampling Layout Design. (See also SOP, Appendix B.1).

a. Selection of study sites. The study sites are selected in extensive saltcedar stands, to allow space for the *Diorhabda* beetles to disperse into an area of several miles. Such areas usually can be found only in strips of varying widths along streams or lakeshores. Also, areas not prone to flooding are preferred since the larvae pupate and the adults overwinter on the soil surface and could drown if flooded. For initial establishment, areas of simple habitat are preferred to avoid aphids etc. more common in complex vegetation that would attract predators that could destroy incipient *Diorhabda* colonies. An initial establishment at Lake Thomas, ca.

30 miles to the northeast where the study originally was planned, was lost probably to native insect predators.

The site selected at the Higgins ranch each of Big Spring meets these qualifications, except that one side of the site floods to a few inches deep, while the larger area does not, giving the opportunity to compare populations in these areas. If the site becomes inaccessible or unusable (highly unlikely) then the sampling program will be moved to Sulfur Draw off Beals Creek, some 10 miles to the northwest, where beetle releases recently were made.

b. Sample unit. The sample unit will be a quadrat of 16 m^2 (4 m in the side) of saltcedar canopy foliage, used for sampling populations of *Diorhabda* beetles, other phytophagous and predaceous insects, saltcedar defoliation by the beetles, light interception, air temperature, relative humidity, and canopy temperature. Canopy area covered by saltcedar foliage also will be measured to express insect populations and defoliation per unit of saltcedar area. Canopy area may be measured a) directly by manual measurement of individual trees made from the ground or from low level aerial photography, with ground truthing to identify the saltcedar trees or b) calculated as the mean of 1 m terminal branch subunits.

Sampling of arthropod populations and defoliation of *Tamarix* by *Diorhabda* will be stratified at three levels of the canopy. Three branches selected at random from the bottom, three from the middle, and three from the top of the canopy will be used as subsample units for estimation of populations and degree of defoliation caused by *Diorhabda*.

The sampling plan at the Higgin's Ranch near Big Spring for the first and second years of the project will consist of 5 transects radiating from the center point of beetle establishment. At the beginning of the growing season of the first year, 3 quadrats will be located in each of the transects. Quadrats will be added in the transects as the beetle population advances from the original release point. The quadrats will be separated approximately by 30 m but the separation can be larger in areas where there are gaps with no saltcedar. Figures B1-6, 7 show distribution of the transects on the study area and location of the 20 quadrats used as sample units during the 2005 growing season. In the 2006 growing season, the same 5 transects will be used for data collection. New quadrats will be added as the beetles disperse toward Beals Creek. When the beetles reach the creek, 2 new transects, one in each direction along the creek, will be demarked. The transects will extend outward to beyond the expected dispersal distance of the beetles each year, based on previous experience at Lovelock, NV, Pueblo, CO and Lovell, WY, which was a radius of 100 m the second year after release, 1 km the third year, and 10 km the fourth year. During the 2007 growing season, the transect along Beals Creek will be extended upstream and downstream for a distance of 10 km from the release site. Distance between quadrats in years 2006 and 2007 will be distanced according with the observed dispersal speed of the beetles.

Defoliated branches will continue to be sampled in order to include continuing defoliation in the sample, and regrowth following defoliation. Regrowth at the base of defoliated trees (Fig. B1-8) will be similarly sampled, 5 subsamples (1-m branch equivalent) per 4 X 4 m quadrat.

Data based on 1 m long branches will be extrapolated to numbers per quadrat by counting the total number of 1 m long branches (or branch equivalents of small branches) present at each level (top, middle, bottom) in each quadrat and multiplying the average of the 3 branches counted by the total number of branches in the quadrat.

c. Critical information. Only one method of on-site/ground level sampling of beetle populations and dispersal is critical to construction of the model. Several methods will be used initially to determine which is most appropriate (discussed in Section B2). Limited experience indicates that to measure populations, the counts of beetles and predators on 1 m long branches provides all the information needed and, although not rapid, is doable. For estimation high levels of defoliation, remote sensing is unsurpassed but cannot be done with sufficient frequency to describe a rapidly moving wave effect. For intermediate levels of defoliation, or frequent estimates, more practical methods would be categorical visual estimations of defoliation on each of the .9 sample branches per quadrat or of entire trees or quadrats (rapid but not very quantitative) or photographic measurement of color separation of each sample branch (more time consuming and more quantitative, but the technique is not yet completely developed). These methods will be evaluated during the first year.

The rapid method for measuring dispersal is to walk the extended transects making visual examination for adults and/or large larvae, then 2-minute counts to roughly estimate beetle density but this is not critical to construction of the model. A better rapid detection system is to use pheromone traps. These have been developed for the *Diorhabda* China/Kazakhstan ecotype and used very effectively at Lovelock and Schurz, NV; however, this pheromone seems to be ineffective in attracting the Crete ecotype and further research is required.

d. Sources of variability. As in all open-field biological research, numerous sources of variability may influence the results. Major anticipated sources anticipated in the present study are weather/climatic, predator populations, condition of the saltcedar trees as influenced by other competitive insect herbivores (exotic leafhoppers or scale insects introduced years ago that often damage the trees), and habitat complexity that may influence predator populations. All these variables are being measured in the present study. The weather station located centrally in the release area measures temperature, humidity, rainfall, wind speed and direction, and solar radiation. A portable temperature/humidity sensor (HOBO) is placed within a saltcedar tree in each quadrat and several under litter under the trees where the larvae pupate. Vegetation species, density, size and canopy cover is measured along the transects each 1 or 2 years before, during and after control. Predator and insect competitor populations are measured as the *Diorhabda* beetle populations are measured.

Also, less frequent monitoring will be made at different locations where the beetles become established, such as at Sulfur Draw, Lake Thomas and Seymour as time, funding and personnel permit.

e. Collection and preservation of samples. In this study, most measurements are made in situ. Plants and insects are collected for identification or for voucher specimens, kept at

the ARS Temple laboratory, with representative specimens sent to the ARS Systematic Entomology and Systematic Botany Laboratories, Beltsville, MD and the museums of the Departments of Entomology and Botany, Texas A&M University, College Station, TX. Identification of spiders and ants will be made by Dr. David Richman, New Mexico State University, Las Cruces, or other specialists.

Plant specimens will be mounted on standard herbarium cards, and insect specimen will be pinned or soft bodied specimens preserved in vials of 95% alcohol, all labeled with the name of the collector, project name, location collected, and identification. Specimens of saltcedar cannot be identified morphologically and will be preserved in alcohol and sent to John Gaskin, ARS-Sidney, MT for identification of species or hybrids, and filed in appropriate botany collections (at GSWRL and other), and recorded on Forms B–18, 19.

Most of the identifications of insects, plants and birds can be made on-site by the ARS biological technicians. Any samples they cannot identify are sent to the experts named above. These identifications can be obtained within 1-2 weeks if important to the ongoing sampling program.

f. Resolution of site or data collection problems. When problems occur with the condition of the site, the data collections, with laboratory tests, etc. the person observing the problem will report it as soon as possible to the ARS Quality Assurance Manager and he to the TSSWCB QA Officer. Minor problems will be resolved by the ARS Manager and major problems in consultation between the ARS manager and the TSSWCB QA Officer.

The spatial distribution of transects and quadrats is not random. The direction of the transects and the location of quadrats on the transects are dictated by the spatial distribution of *Tamarix* in the study area and by the convenience of having quadrats located at approximately equal distances. The non random distribution of sampling units in spatial studies is very common and is not a limitation for the application of probabilistic or statistical methods that require random variability since the attributes measured in the sample units, such as insect populations, degree of defoliation, saltcedar characteristics, micrometeorological factors, etc., are continuous random variables. The random selection of sample subunits (branches) within the canopy of every quadrat is another source of random variability required for the application of probabilistic and statistical methods.

An adequate number of sample repetitions is required to make objective inferences about the effect of spatial and temporal variability on the dispersion of *Diorhabda*, and on the degree of saltcedar control by the beetle. The horizontal spatial variability effects will be estimated with a number of quadrats located along transects that will cover the saltcedar stand area colonized by *Diorhabda*. The vertical spatial variability effects will be estimated with 9 branches, 3 from each canopy level (top, middle and bottom). The temporal variability will be studied with weekly sampling during the growing season. The number of quadrats and distance between quadrats along each transect cannot be pre established since they depend on beetle population size and how fast the insects move from tree to tree along the transects. The estimation of parameters for the deterministic models (Kovalev's and Okudo's) require the development of several waves of

insect population as a function of distance (Figure A6-1, page 25). Quality of parameter estimation for the spatial multiple regression model will depend on the number of quadrats along the transects.

At the end of the first growing season, in 2005, we had 3 transects with 6 quadrats each, one with 4 and one with 1 quadrats. This number of sample repetitions are expected to be adequate for model parameter estimations and to account for the horizontal and vertical spatial variability that affect *Diorhabda* population and its dispersal. Sampling was carried out during 18 consecutive weeks which will be adequate to estimate temporal effects over *Diorhabda* dispersal and saltcedar defoliation. Experience at other locations shows that the area colonized by *Diorhabda* increases dramatically as population increases from year to year but the number of sample units should remain at a manageable number with the limited human resources available. We believe that 25 quadrats is around the maximum number of sample units that we can manage. Larger areas in future years will be sampled using quadrats separated by larger distances. We may get reduced to only one transect after the beetles reach Beals Creek and all the 25 quadrats would be located along that transect.

Section B2. Data Collection for the Models: Measurement of Model Parameters

A. Canopy Cover of Saltcedar

1. Within quadrats.

We will calculate the canopy area (as seen from above each tree) within the 16 m^2 quadrats. The canopy area will be measured in late May of each year (after spring growth is completed) and again, if necessary (trees have died or grown), in September. This ground-level canopy measurement will be correlated with measurements from low-level aerial photography and extrapolated to the entire study area (Form C-15). Unknown plants will be sent to taxonomic specialists for identification (Form C-19).

The density canopy cover will be calculated as follows:

$$cc = \sum_i \Pi r_i^2 \qquad [19]$$

cc: Canopy cover area.
i: 1,2,3,.....n # of trees
r: average radius of tree i.

A diagram from the projection of the canopy on the ground will be done for each quadrat. Radius of the different trees will be record to calculate the canopy cover area *cc* using equation 17

$$ccd = \frac{cc}{16} * 100 \qquad [20]$$

Where ***ccd*** is canopy cover dentity, ***cc*** is canopy cover area in m^2 calculated with equation 17. 16 is the area of the quadrat in m^2.

Vigor will be visually judged and recorded (Form C-3, 8) with a scale from 1 to 5, where 5 will be the value assigned to a completely healthy foliage and 1 to dead foliage. Surveyors will be trained to make sure that each scale value means the same for all of them.

2. Landscape scale.

Average % defoliation (not defoliated, % partially defoliated, 95%+ defoliated, % regrowth dead) will be determined from the ground-level assessments of 1-m^2 branches extrapolated to each 16 m^2 quadrat, then extrapolated to the area of the aerial photographs needed in the model for characterizing the advancing wave of defoliation (recorded on Form C-15).

B. *Diorhabda* Beetle Density

At each quadrat along the transects, *Diorhabda* beetles will be counted on the nine 1 m long terminal branches, 1 to 3 branches per tree depending on the size of the tree, using branches that occur within the 4 X 4 m quadrat; the branches will be permanently marked and sampled repeatedly throughout the growing season. Number of egg masses, larvae at first, second and third instar, and number of adults will be counted and recorded on Form C-3. The total number of branches inside the quadrat will be counted and recorded in the same data collection format.

Population density of *Diorhabda* at a given stage in a stratum of a quadrat will be estimated using the following equation. The number of branches in each canopy stratum will be counted to get the term b in the equation. The length of branches in each canopy stratum will be estimated measuring a sample of 20 branches per canopy stratum to obtain the term l in the equation.

$$N_s = c\, l\, b \qquad [21]$$

s: Bottom, middle, top.
N_s: *Diorhabda* population density (insects per meter of branch) in a given stage.
c: Mean number of insects on 1 m of 3 branches.
l: Mean length of branches in the canopy stratum.
b: Number of branches in canopy stratum.

Total estimated population density will be obtained by averaging the estimates for top, middle, and bottom branches, calculated using equation [21].

Branches will be selected 3 in the top 1/3 of the tree, 3 in the middle third and 3 in the bottom third, and equally distributed within the three 120° sections of the quadrat. Branches in the top part of the tree will be sampled from a 10 ft stepladder. The transect number, distance along the transect, tree number, branch number, and position on the tree will be recorded on the field record forms.

Defoliated branches will continue to be sampled in order to include continuing defoliation in the sample, and regrowth following defoliation. Regrowth at the base (Fig. B1-8) or upper branches of defoliated trees will be similarly sampled.

Since the growth form of saltcedar trees is such that the upper branches are abundantly sub and sub-sub branched, the middle branches less so, and the lower sparcely so, a weighting factor will be extracted based on the total length of all sub and sub-sub branchlets per 1-meter-long branch (Form C-10). Foliage density and beetle populations will be extrapolated to an area basis by counting all 1-m branches in each strata in each quadrat once a year (Form C-9).

Beetle populations may be extrapolated to numbers per m^2 over the landscape area in the remote sensing images (as done for canopy defoliation, in paragraph A.2, above) of known areas (Form C-16).

C. Saltcedar Defoliation by *Diorhabda* Beetles

Estimating the amount of defoliation of saltcedar produced by the *Diorhabda* beetles in the early stages of defoliation is difficult and complicated by several factors. In the later stages, based on previous experience at Lovelock, NV and Pueblo, CO, defoliation reaches nearly 100%. Defoliation by the beetles, at this stage, overrides all other factors, and measuring defoliation becomes very simple, i.e., defoliation equals the previously measured canopy cover. However, following defoliation, the saltcedar plants resprout and measurement again becomes complicated. We will use several methods, each helping to calibrate the others (Form C-17).

1. Visual estimate.

This method will be used to estimate percentage of green, yellow, brown, and dead foliage. Pecentage of damage by *Diorhabda* and by leafhoppers will also be recorded as is shown in Form C-3. Whether the estimates are done in regular or regrowth foliage will be recorded in the data formats. This is a method commonly used in estimating cover of rangeland plants in experimental plots; with practice and reference standards it can approach most other methods in accuracy and is much faster. Photographic reference standards will be used as discussed in (a) above. The percentage of defoliation will be placed visually into categories: 0-10%, 10-30%, 30-70%, 70-90% and 90-100%, estimated separately for beetle defoliation, healthy green foliage, and damage by other insects or seasonal senescence (Form C-3).

2. Photography of 1-m terminal branches.

The branches will be selected as described for "Sampling Beetle Density", above, and will use the same branches used for the beetle counts. Each branch will be photographed in color on each count date using a 4.1 megapixel (or better) digital camera, with a white background card held behind and against the branch that is graduated in 5 cm grid lines. The photographs will be made from the same distance, and so are to the same scale. The background card will bear the transect #, quadrat #, tree #, branch #, date and time, which will appear in each photograph.

The digital images will be color-separated using specialized software and the area of green and brown foliage measured. Reduction (or increase) in foliage from the previous week's measurement equals the amount of defoliation or regrowth (Form C-10).
Complications of this method are that a) some overlapping of foliage occurs so foliage area is underestimated to varying degrees. To correct for foliage overlap, a set of branches outside the sample area will be measured photographyically and the branch will be removed, tagged and then compared with the same branch measured in the laboratory on a Li-Cor leaf-area meter where all foliage is removed so that overlapping does not occur. A set of reference photos will

be prepared for use in correcting the measured green area of the sample branches (the brown foliage usually is so sparse that little overlapping occurs). b) Foliage yellowing through natural senescence or from attack by *Opsius* leafhoppers or *Chianapsis* can be measured by color separation but death or missing foliage probably cannot.

3. Biomass. The foliage measured by the Licor leaf area meter (see Paragraph C.1 "Photography---") above, will be placed in paper bags and dried in an oven (specialized drying ovens are available a Temple) and used to standardize (a) and (b), above. For saltcedar biomass estimation three salcedar branches will be collected from each canopy section at each sample quadrat and at each sampling time. Samples will be taken to the laboratory for oven drying and weighing. Wood weight of branch samples will be discounted for the biomass estimate of the branch. Dry weight of branches is recorded on Form C-13. Using the total length of branchlets per 1-m branch for each stratum (Form C-9), and total number of branches per canopy section (Form C-8), an estimate of biomass per canopy section and for the total canopy in the quadrat will be obtained.

4. Light interception. Radiation interception by the saltcedar canopy can be a factor that affects dispersal of *Diorhabda* through its direct relation to food amount available or its impact in canopy micrometeorology. Measuring will be done at five sites in each sample quadrat following the method described above in the section titled 'Light bar' within 'Sampling saltcedar defoliation'. The degree of light interception by the canopy will be used also as one of the methods to estimate defoliation. A Decagon 1-meter long light bar will be used to measure light intercept of each entire sample plant in the field. The light bar is inserted into the lower third of the growing plant at 10-20 locations and the average reading recorded, compared with an open-sky reading beside the plant at the same time (Form C-11). Readings are made between 10:00 am and 3:00 pm on sunny days. Readings of all plants will be made in late winter before foliage appears (this measures light interception by the stems), in April or May after foliage is produced but before beetle defoliation, and at each sampling date (measures defoliation and regrowth). This method is complicated by defoliation by other insects and natural senescence, but can be corrected as in (a), (b) and (c) above.

5. Temperature of foliage. Canopy temperature may be a factor linked to the distribution of the beetles in the salcedar canopy, it will be measured with an infrared temperature meter. Three instantaneous readings at sampling time and at each canopy section will be done and recorded.

6. Remote sensing. The site will be photographed by low-level aircraft using 9 inch aerial film, flown at 500 ft altitude above the ground to provide very high resolution. This will be done in mid-to late September to capture nearly all defoliation occurring during the year (Form C-15). Previous sampling at Lovelock, NV has shown that this method is highly accurate in providing a wide-scale, rapid measure of defoliated saltcedar, which is very distinct from the green saltcedar and other green vegetation. Ground truthing will distinguish the green saltcedar from other green vegetation until hyperspectral sensing technology is capable of making this distinction. Computer software analysis of the images can provide an accurate measure of

saltcedar canopy cover. This will provide a calibration of the above defoliation estimates and of the entire model in predicting the rate of dispersal (defoliation) produced by *Diorhabda*.

From the five methods described above, the remote sensing is the most accurate in the late stages of defoliation, when entire trees are defoliated and the defoliated trees are concentrated in patches or cover large areas. In early to intermediate stages of defoliation we need to rely on the ground methods. From the ground methods the one based on branch photographs is expected to be the most accurate, it is also the most time consuming. A regression based calibration of the light-bar method and the biomass method against the photograph method may yield a method that is easier and faster to estimate saltcedar defoliation by *Diorhabda*.

D. Sampling Other Biotic Factors

Other biotic factors that will be sampled because they are considered as possible explanatory factors of *Diorhabda* dispersal and degree of defoliation caused by *Diorhabda*, are populations of *Diorhabda* predators, populations of other phytophages that feed on saltcedar, saltcedar biomass, saltcedar canopy cover, and saltcedar vigor. The sampling of *Diorhabda* predators and other phytophages that eat saltcedar will follow the same sample system as the *Diorhabda* counts and will be recorded (Form C-2). Damage caused by leafhoppers and other phytophages, will be estimated as in Paragraph C.1, above. Unknown insects, mites and spiders will be sent to taxonomic specialists for identification (Form C-18).

1. Leafhoppers. Populations of the exotic, saltcedar-specific leafhopper, *Opsius stactogalus*, often reach levels sufficient to cause yellowing of the foliage and even defoliation, and may compete with *Diorhabda* for the food resource. Populations at low densities will be counted, and at higher densities by estimated groups of 5 or 10 leafhoppers on the 1-m long branches. Unknown insects, mites and spiders will be sent to taxonomic specialists for identification (Form C-18).

2. Scale insects. Populations of the exotic, saltcedar-specific scale, *Chionapsis eutrusca*, occasionally reach damaging levels. Populations will be estimated by counting in groups of 5 or 10.

3. Other phytophages. Several native generalist phytophagous insect species sometimes feed on saltcedar but rarely cause noticeable damage except occasionally by grasshoppers. Populations will be estimated by counting individuals on the 1-m long branches.

4. Predators. Predators usually are found in association with *Diorhabda*, leafhopper or scale insects and may severely reduce *Diorhabda* populations. Principal predators are spiders, assassin bugs, ladybird beetles, ants and occasionally others. Individuals of each species on each 1-m branch will be recorded and the prey species will be noted when observed. Twice annually (June, late August) the effect of predators to *Diorhabda* eggs, larvae and pupae will be determined by comparing losses during these stages in caged vs. uncaged eggs, larvae or pupae

(Form C-7). Also, laboratory tests will demonstrate the ability and extent of feeding by the major predator species on the *Diorhabda* stages.

5. Parasites. Samples of 10 of each stage (egg, 1st, 2nd, 3rd, and adult) of *Diorhabda* beetles from 3 quadrats with moderate to high beetle populations will be collected at each count date, returned to the Temple laboratory, and held in clear plastic boxes with saltcedar foliage until they reach the next stage (eggs to 1st instar larvae; larvae to adult), when the number of parasites will be recorded (Form C-6).

E. Sampling Abiotic Factors

Canopy temperature, air temperature, relative humidity, and radiation intercepted by the saltcedar canopy will be measured in every quadrat with the purpose of identifying factors that may affect the dispersal of *Diorhabda* and the defoliation of saltcedar by the beetles (Form C-14). The continuous effect of temperature and relative humidity will be studied using air temperature and relative humidity recorded by a Hobo sensor placed at shoulder height in a saltcedar tree in each quadrat and data loggers.

Other abiotic factors that will help to explain the speed and direction of *Diorhabda* dispersal are wind direction, wind speed, air temperature, relative humidity, rain, solar radiation, and litter temperature (Form C-14). Litter temperature is measured by sensors buried under the litter at three quadrats that have three different degrees of foliage density. Litter temperature is one of the variables that is expected to influence survival of *Diorhabda* through the winter. Another of the variables critical for the survival of the beetles through the winter is flooding which will be measured with water level sensors located at different points in the study area. Area weather characteristics are especially useful for inter-location comparisons. Area weather factors will be measured and recorded by a Campbell weather station located in a fenced area, unlikely to flood, in the center of the study area.

F. Sampling SOPs and Training of Personnel

Sampling SOPs will be provided to all personnel engaged in taking the samples. The personnel will be interested in how to make each type of sample, along with side by side practice in the field with the trained ARS technicians (see Appendix B).

G. Sampling Equipment and Instrumentation Needs

- Campbell Weather station (temperature, RH, rainfall, wind speed and direction, solar radiation, remote litter/soil temperature sensors) (2 ea, on hand)
- HOBO small temperature/RH recorders (20 ea)

- Laptop computer, 1 ea (on hand)
- Light bar, 1-m long, Decagon Model Accu PAR LP-80, 1 ea (on hand)
- Tape measure, 100 m (on hand)
- Compass (on hand)
- Insect sweep nets (on hand)
- Stepladder, 4 ft, 8 ft and 10 ft aluminum
- Dissecting microscope (on hand)
- Hand clippers and loppers (on hand)
- Clipboards, rain protected (3 ea) (1 on hand)
- Camera, digital, 35 mm, Canon EOS-20D, 8.0 mega pixel (1 ea) (on hand)
- Leaf-area meter, Licor Model LI-3000A (1 ea) (on hand)
- Scanner for 9" aerial photographs, Epson Model Expression 1680 (1 ea) (on hand)
- Image spectral separator (1 ea) (available at ARS Weslaco)
- Aircraft w/aerial photographic cameras (available at ARS Weslaco)
- Nylon sleeve bags
- Insect field cages, 10 X 10 X 8 ft high, aluminum frame w/saran screen
- Temperature/humidity meter, hand held, infrared, Extech Model RH-101

Section B3: Sample Handling and Custody Requirements

Data collected from all field monitoring and sampling, and from all laboratory tests, is recorded on field or laboratory data forms as it is being collected (Forms C-2 to C-17). All data sheets are labeled with type of data, date, site name, quadrat and transect number, tree number, branch number and location, and person(s) collecting the data. Data sheets will be held on field clipboards during collection, transferred to labeled data-sheet binders immediately after each collection trip, and stored at the ARS Temple laboratory for entering into computer data bases and analyzed at the end of the growing season.

Samples collected in the field for identification or further study, such as branches for area meter or for biomass measurements will be labeled with tags bearing the date, location and sample number. Insect or plant specimens sent to other locations for identifications are assigned a specimen number that is attached to the specimen and recorded in a log book maintained at the ARS Temple laboratory (Forms C-18, 19).

Voucher specimens of the various insects and plants sampled, with their date and location of collection will be maintained by ARS at GSWRL, Temple, Texas. These will include:

a) The control insect (*Diorhabda* leaf beetles)
b) Parasitoids or predaceous insects and spiders of *Diorhabda*
c) Herbivorous competitor insects (leafhoppers, scales, others) of *Diorhabda*
d) Plant specimens of saltcedar and native plants

The soft-bodied insects and spiders, and insect larvae will be transferred to vials of 70% ethanol, and hard bodied insects, moths and butterflies will be killed in a killing jar and transferred to glassine envelopes, and beetles and other hard-bodied insects will be killed and placed in dry vials for return to the laboratory. Hard-bodied insects will be preserved on insect pins and held in an insect storage cabinet. A few identified voucher specimens will be labeled with data and location collected, host plant, insect name and identifier and retained at the Temple laboratory for future comparison with other field collected insects. Each sample will be identified by sample number, site name, date of collection, plant species, tree number (tagged and GIS located), and collector's name. Field record sheet also will record this information and weather conditions and time of day collected.

Section B4: Identification of Insect and Plant Samples

Insect samples will be pinned and plant samples will be glued to herbarium sheets and labeled (name of collector, date and location of collection) according to standard procedures. Identification will be made by skilled ARS or Texas A&M entomologists and botanists. Questionable specimens will be made by the ARS Systematic Entomology Laboratory, Beltsville, MD or other recognized taxonomic authorities at Texas A&M University; New Mexico State University, Las Cruces; or University of Texas, Austin, as appropriate. Identified voucher specimens will be preserved by ARS at GSWRL, Temple, TX for comparison and identification of future collections (Form C-18).

Plant samples, including the various hybrids of saltcedar, will be handled in a similar manner and identified by DNA analysis by ARS, Sidney, MT (Form C-19).

Section B5: Quality Control Requirements

The sampling and monitoring conducted in this biological control project involve identification of the insect and plant species and the measurement of insect populations and dispersal, factors causing mortality of the control insect (measurements of parasitism, predation, and competition from exotic leafhoppers and native insects). Most of this is done by *in situ* counting the various insects on the plants, categorizing the damage to the plants, and measuring plant size and canopy cover along transects. Plant branches are removed and returned to the ARS laboratory at Temple for measurement of leaf area with an area meter or at Temple for drying in ovens to determine biomass. The area-wide degree of defoliation and recovery of native vegetation are measured by remote sensing.

All these methods are probability based and can be analyzed statistically. They are designed to compare the density, cover and abundance of saltcedar, before, during and after control, as the wave of beetles and defoliation passes through a saltcedar stand over a 3-year period.

To assure accuracy in the data collection, the field technicians are taught to identify the different life stages of the beetles by demonstrating the distinguishing characters under a dissecting microscope in the laboratory until they can readily distinguish the *Diorhabda* beetles. Identification will be based on photographs and/or drawings of the identifying characteristics of specimens and by comparisons with identified, pinned *Diorhabda* beetles, or immature stages preserved in vials of alcohol that have been identified by taxonomic specialists. To assure accuracy, the counts made by the field technicians will be compared immediately afterwards by the field biologist by the experienced ARS technicians until the field technician counts are within 10% of the counts made by the experienced ARS personnel at the same time and location.

Laboratory identifications will be made by ARS technicians Robbins and Tracy; all unfamiliar specimens will be sent to taxonomic specialists at the ARS Systematic Entomology Laboratory (SEL), Beltsville, MD; New Mexico State University (NMSU), or Texas A&M University (TAMU), for identification. Voucher specimens will be maintained at the Temple ARS laboratory. Accuracy of the field identification of plants and insects by the ARS technicians (Robbins and Tracy) will be determined by comparison with insects from the same collections (location and date) made by taxonomic specialists at SEL, NMSU, TX A&M, or the University of Texas, Austin.

The accuracy of the aereal extent of canopy defoliation of saltcedar will be assured by several complementary methods, each of which is used to calibrate the others: a) visual estimation of present defoliation of 40 cm branches or whole trees using standard reference photos of the different percent defoliation categories determined from b and c), b) leaf area measurement and c) dry-weight biomass determination of 40-cm branches, d) photographic spectral separation of damaged vs. healthy foliage on 40 cm branches, e) light-bar measurements of foliage interception of light by the whole tree in the field, and f) remote sensing of defoliated vs. healthy saltcedar canopy by low-level aircraft imagery (see SOP and Forms in Appendix B).

Section B6: Equipment Testing, Inspection, & Maintenance Requirements

A. Testing, Inspection, Maintenance

Manufacturers' recommendations for scheduling testing, inspection, and maintenance of each piece of equipment will be followed or exceeded. All equipment testing, inspection and maintenance will meet the requirements specified by the EPA. Maintenance and inspection logs will be kept on each piece of field and laboratory equipment; general maintenance checklists will be filled out for sampling equipment prior to each sampling event and serviced as needed. A general maintenance (GM) sheet will be filled out for all sampling equipment during each GM inspection. The GM sheet contains a check list for all equipment and routine maintenance activities will be performed and recorded on Form C-23. Any equipment, which needs attention, will be serviced during the presampling inspection, with all additional activities described in the comment section. Any maintenance or other required activities that can not be completed during the scheduled GM inspection will be reported to the field supervisor, who then arranges for resolution. The field supervisor checks the presample GM sheets and schedules additional follow-up to ensure that any problems or potential problems are resolved as soon as possible.

To minimize downtime of all measurement systems, all field measurement and sampling equipment, in addition to all laboratory equipment, must be maintained in a working condition. Also, backup batteries or common spare parts will be made available if any piece of equipment fails during use so that repairs or replacement can be made quickly, allowing measurement tasks to be resumed. All staff who use chemicals, reagents, equipment whose parts require periodic replacement and other consumable supplies receive instruction concerning the remaining quantity (unique for each supply) which should prompt a request to order additional supplies.

Equipment used for these experiments consist of HOBO temperature/humidity recorders and HOBO rain gauges located at the field sites. At least once a month, one of the ARS Project Technicians (Tracy or Robbins) will download the data into a notebook computer and examine the equipment for proper functioning. A spare of each type of unit is brought to the field site from GSWRL, Temple and if the installed unit is malfunctioning it is replaced by the spare and the malfunctioning unit is returned to the Temple laboratory for servicing, adjustment or returned to the company for repairs if needed. A log will be maintained at the Temple ARS laboratory of the accuracy checks and the calibration performed (Form C-22).

B. Field and Laboratory Equipment List

(See Section A2.G and Section B2.G). Location of equipment used in this project will be recorded on Form C-21.

Section B7: Instrument Calibration and Frequency

All instruments or devices used in obtaining environmental measurement data will be used according to appropriate laboratory or field practices.

All instruments or devices used in obtaining environmental measurement data will be calibrated prior to use. Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. All calibration procedures will meet the requirements specified in the USEPA-approved methods of analysis. The frequency of calibration recommended by the equipment manufacturer, as well as any instructions specified by applicable analytical methods, will be followed. All information concerning calibration will be recorded by the person performing the calibration and will be accessible for verification during either a laboratory or field audit (see Form C-22).

All calibration procedures used in the field or laboratory will meet or exceed the calibration frequencies published in the test methods used for this project. Additional calibration procedures may be conducted if laboratory personnel determine additional calibration is warranted as beneficial to this project.

The HOBO temperature recorders and rain gauges will be calibrated at the Temple ARS laboratory annually during the off-season, according to manufacturers directions. Accuracy will be verified monthly in the field. The HOBO temperature recorders will be calibrated by comparison with a certified thermometer and the rain gauges by pouring a measured amount of water into the rain gauge.

The light bar is calibrated by the manufacturer when purchased and each 3 years when returned to the manufacturer for servicing. Accuracy also is compared with a second light bar owned by the Temple ARS laboratory. The light measurements made are relative measurements, being the difference between the measurements under each tree and the measurement immediately thereafter of the open sky beside each tree. Therefore, inaccurate calibration does not affect the accuracy of the relative measurements.

Section B8: Inspection/Acceptance Requirements for Supplies and Consumables

All supplies and consumables received by ARS are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements and recorded (Form C-24). Labels on reagents and chemicals are examined to ensure they are of appropriate quality.

Supplies are inspected by ARS Technicians Tracy or Robbins when received at the ARS Temple laboratory.

Section B9: Data Acquisition Requirements (Non-direct Measurement)

Determinations at sampling sites will be based upon data collected during the time frame of this project. However, data collected from other state or federal projects will be used as supplemental information to meet data quality objectives (see Section A7). In determining biological parameters at sampling sites, data collected prior to this project's initiation will be used to provide some of the pre-infestation data used for pre- and post-benefit comparisons.

The data collected under other projects will be referred to as historical data; this will supplement data from this project in the assessment of changes in vegetation composition, and water conservation.

Data from SC/BC projects at other locations in the western U.S. are used for comparison of *Diorhabda* beetle response and control produced in different climatic/ecological zones.

Agreements also have been obtained for using data from releases made in May 2001 at Lovelock and Schurz, NV, Delta, UT and Pueblo, CO (sites with Fukang/Chilik beetles well established) and at Lake Meredith in northern Texas. Thus, data is available from sites where releases are planned during 2005, and from sites where releases were made last year or 5 years ago. This data from where dispersal has already been in progress is valuable in constructing the model, since it is equivalent to observing one site for 5 years.

B10: Data Management

A. Field Collection and Management of Routine Samples

Field staff will visit sampling sites on a weekly to monthly basis to collect data on the control beetles and of their effects on the saltcedar plants. Site identification, date and time, personnel, measurements of field parameters, and any comments concerning weather or conditions at the site are noted on a field data sheet. Field log book (Form C-1) and field data sheets are filled out on site for each location visited (see examples of field data sheets in Appendix B.B).

Data from the field is recorded by pencil on printed paper record sheets and held during record taking in rain-protected clipboards. At the end of each day, these are transferred to labeled folders in a portable file kept in the vehicle. After each field trip, the records will be hand-carried to the ARS-Temple laboratory, along with any record sheets completed by on-site personnel. At Temple, the data sheets are filed immediately in record notebooks, each type data under a separate tab, held securely together with metal brackets, each notebook will be stored at an identified location on shelves in the laboratory.

Records will be transferred to an electronic data system, with CD backup, during the week following collection or as soon as possible thereafter. CD's will be labeled and filed in a CD container in a different laboratory room at ARS-Temple. Back-up CDs for the model development will be stored in a labeled CD container at the TAES building, at Temple (see Form C-16).

Specimens of insects and plants are assigned a unique sample identification from a log book, a label with that number is placed on the specimen and recorded on the COC forms, and sent to taxonomic specialists for identification. When the identified specimens are returned, the correct name is recorded on the COC form and the specimens are stored in the plant herbarium or insect collection of ARS at Temple, TX. COCs are kept in three-ring binders in the ARS office for at least five years.

Field data and species identification will be verified by field personnel and/or a data analyst. As field sampling is completed, laboratory personnel will enter the results from laboratory notebooks into EDAS database. The Project Biologist will be responsible for verifying that data in the EDAS database match the data in the laboratory notebooks. After verification has been completed on all data for a group of samples, the laboratory manager will notify the data analyst that a group of data is ready for review. The data analyst will check for abnormalities or problems by examining all field, and laboratory data. Site names, appropriateness of data values, completeness of data, dates and times, container numbers, comments and all other data will be reviewed within the EDAS database. Any questions or abnormalities will be investigated, relying largely on field data and general maintenance sheets, field biologist, laboratory notebooks, and laboratory personnel. As appropriate, corrections will be made to the EDAS database with appropriate documentation maintained.

B. Backup and Disaster Recovery

The electronic data, along with the model and results generated by the model, are backed up daily onto an alternate device (i.e. – CD, or comparable media) (Form C-16). In the event of a catastrophic systems failure, the media can be used to restore the data. Data generated on the day of the failure may be lost, but can be reproduced from raw data in most cases.

C. Archives and Data Retention

Original data recorded on paper files are stored for at least five years. Data in electronic format are stored on alternate media and/or drives in a climate controlled, fire-resistant storage area at ARS, Temple, TX.

Field data sheets are kept in covered clipboards in the field and transferred to binders each night. These data sheets are placed for 5 years in categorized and labeled binders at GSWRL, Temple by technicians Tracy and Robbins. Each year, as time permits, the data are transferred to a computer database.

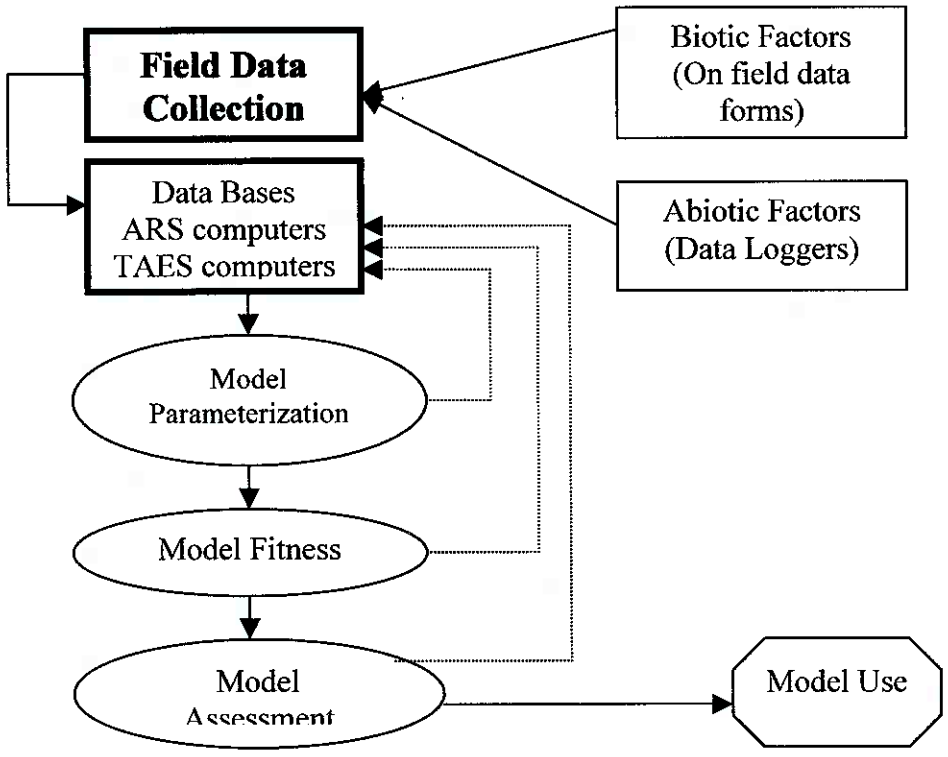


Figure B10-1. Information dissemination diagram.

Section C1: Assessments and Response Actions

The commitment to use approved equipment and approved methods when obtaining environmental samples and when producing field or laboratory measurements requires periodic verification that the equipment and methods are, in fact, being employed and being employed properly. This verification will be provided through an annual field and laboratory performance audit performed by the QA officer. Individual field personnel will be observed during the actual field investigation to verify that equipment and procedures are properly applied. Any problems that are discovered in the monitoring procedures that would affect the quality of data collected at the demonstration sites will be addressed by the project participants and followed up with a CAR (Form C-26). Follow-up observations will occur within three months when discrepancies are noted. Also, TSSWCB and EPA will conduct yearly performance audits for this project.

All analyses of field data will have the precision and accuracy of data determined on the particular day that the data were generated.

To minimize downtime of all measurement systems, all field measurement and sampling equipment, and all laboratory equipment must be maintained in a working condition. Also, backup equipment or common spare parts will be available if any piece of equipment fails during use so that repairs or replacement can be made quickly and the measurement tasks resumed.

The main mechanism for assessing the performance of the models used will be evaluation of their goodness of fit with respect to the observed field data. In case of unsatisfactory goodness of fit, adjustments in the parameterization will be performed. Independent data subsets will be used for the development of the model parameters and for the input of the models that will be employed for their assessment.

Section C2: Reports to Management

Quarterly progress reports will note activities conducted in connection with the sampling of field populations of *D. elongata* beetles and their effects on saltcedar and of the plant and wildlife monitoring. Items or areas identified as potential problems , and any variations or supplements to the QAPP will be noted. Corrective action report forms will be utilized when necessary (Form C-26). CARs will be maintained in an accessible location for reference at GSWRL Temple. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP. The quarterly reports will also inform about performance of the models fitted to field data that has been collected up to the reported period.

The field measurement and sampling for the project will be done according to the QAPP. However, if the procedures and guidelines established in this QAPP are not successful, corrective action is required to ensure that conditions adverse to quality data are identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correct of identified problem. Corrective Action Reports will be filled out to document the problems and the remedial action taken. Copies of Corrective action reports are included with annual Quality Assurance reports. They will also discuss any problems encountered and solutions made. These QA reports are the responsibility of the Quality Assurance Officer and the cooperating Agency Lead and are available for review upon request.

Section D1: Data Review, Validation and Verification

All data obtained from field and laboratory measurements will be reviewed and verified for integrity and continuity, reasonableness, and conformance to project requirements, and then validated against the data quality objects outlined in Section A7, "Data Quality Objectives for Measurement Data". Only those data that are supported by appropriate quality control data and meet the DQOs defined for this project will be considered acceptable for use.

The procedures for verification and validation of data are described in Section D2, below. The ARS Project Manager is responsible for ensuring that field data are properly reviewed, verified, and submitted in the required format for the project database. The QA officer is responsible for validating that all data collected meet the data quality objectives of the project.

Section D2: Validation and Verification Methods

Quality control aspects of databases include the following:

- Sample data are identified with a unique, sequential sample number.
- Entries into the EDAS database are verified against field data sheets and laboratory notebooks prior to transfer into the EDAS database. This constitutes an on-going internal audit.
- All extreme data outliers will be verified by review of the field data sheets or laboratory notebooks to make sure these points are not transcription errors. If an error is found, the data manager will be notified with the appropriate documentation of the change that is needed in the EDAS databases.
- Unusual circumstances associated with sampling sites or collection of samples are noted in the Comments section of the field notebooks. Comments are copied onto the databases to provide additional information for any questionable results.
- Entries in databases are verified by someone other than the person who enters the data.
- Print-outs of electronically generated data are archived for subsequent verification of data.
- Mistakes in logbooks are crossed out with a single line, corrected, initialed and dated by the person correcting it. This ensures proper lines of communications concerning queries of data validity.
- Very important component of the validation and verification methods are the goodness of fit tests to establish statistical similarity between the model predicted and the field observed values. Lack of fitness can be due to data errors, deficient model parameterization, or inadequacy of the model. All possibilities will be evaluated and corrective measures taken.

Section D3: Reconciliation with Data Quality Objectives

The ARS Project Manager shall be responsible for reviewing raw data and shall check calculations to verify that data are entered into the database correctly and be responsible for internal error corrections. Corrective Action Reports will be initialed in cases where invalid or incorrect data have been detected.

Data completeness in this project will be relative to the number of sampling events. It will be the goal of this project to achieve 90 percent completeness; however, statistical analysis will be the final indicator of data validity.

Representativeness and comparability of data, while unique to each individual collection site, is the responsibility of the ARS Project Manager. By following the guidelines described in this QAPP, and through careful sampling design, the data collection in this project will be representative of the actual field conditions and comparable to similar applications. The Project Manager will review the final data to ensure that it meets the requirements as described in this QAPP.

Any limitations of use of the data, due to climate, predation or parasitism of the *Diorhabda* beetles, capability of the models to reasonably reproduce field performance of *Diorhabda,* or other limitations will be discussed in the reports to other users or in published papers.

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APPENDIX A

Need for and Methods of Controlling Saltcedar – Previous Research on Biological Control of Saltcedar

A. Need for Control of Saltcedar

The invasion by exotic saltcedars, small trees or shrubs from Eurasia, along western U.S. streams and lakeshores has produced one of the worst ecological disasters in the recorded history of the region. The plant was first recorded in a nursery in New York in 1823, and thereafter it was widely planted throughout the West as an ornamental and to control streambank erosion. It had escaped cultivation by the 1890's, was noted as a pest in some areas by 1910, it rapidly invaded riparian areas after the late 1920s, and by 1950 it occupied large areas of many western riverbottoms and lakeshores (Robinson 1965). Today, it occupies ca. 2 million acres of prime riparian bottomlands and it is still spreading along tributaries and small streams. Worldwide, 54 species are recognized, with the centers of origin from central Asia to China and in the eastern Mediterranean area (Baum 1978). Some 10 species have been introduced into the U.S. (Baum 1967, Crins 1989); 4 of them, and their hybrids (Gaskin and Schaal 2002), cause almost all of the damage (reviewed by DeLoach and Tracy 1997, DeLoach et al. 2000, 2003) (Fig. A5-1; Fig. A5-2).

1. Environmental Damage.

Dense thickets of saltcedar have displaced the native plant communities. Saltcedars are heavy water users, lower water tables and cause small streams and desert springs to dry up; they increase soil and water salinity, increase wildfire frequency, and reduce recreational usage of parks and natural areas. They alter stream channel structure, cause bank aggradation, narrowing, deepening and blockage of channels, and alter water quality.

These changes to the plant community and to the physical environment combine to severely degrade wildlife habitat. The native wildlife (mammals, birds, reptiles and amphibians, fishes, insects and other invertebrate) have not evolved with saltcedar and are largely unable to utilize it or to adapt to the environmental changes it produces. Saltcedar foliage is rather unpalatable, its tiny fruits and seeds are not utilized, cavity dwellers and granivores are mostly absent in saltcedar thickets, most native insects are unable to develop on it though many are attracted to its flowers, and the altered aquatic environment is harmful to many fishes, amphibians, and to the species of insects and invertebrates on which they feed. Saltcedar has greatly reduced biodiversity in the majority of the vital southwestern riparian ecosystems. Many wildlife species have declined as saltcedar has replaced the native plants, several have become endangered, and at least 50 T&E species, mostly fishes and birds but including also mammals, reptiles, amphibians, insects and plants have been severely affected (reviewed by DeLoach and Tracy 1997, DeLoach et al. 2000).

The minute risk of damage that might be produced by biological control must be weighed against the great known damage caused by saltcedars, and the risk from the no-action option of allowing this damage to continue, as pointed out by Pimentel et al. (1992) and Pimentel (2000). These dangers are revealed by surveys in Australia showing that more than 50 plant species are endangered because exotic, invading weeds out-compete them (Bell 1983) and in Germany showing that 89 of 581 rare plants are declining because of herbicidal applications to control weeds (Sukopp and Trautmann 1981). In the United States, Stein and Flack (1996) estimated that approximately 400 of the 972 federally listed threatened and endangered species of plants and animals are at risk primarily because of competition with and predation by non-native species. Wilcove et al. (1998) estimated that 48% of 56 imperiled birds and 30% of 641 species of plants in the continental United States are imperiled because of alien species. Recent programs along the Pecos River of New Mexico and Texas seek to eliminate extensive, monotypic stands of saltcedar, using herbicidal and mechanical controls. Although probably safe along the saline Pecos River, such controls are likely to damage native plant and animal communities if extended to other areas of mixed saltcedar-native vegetation unless biological control is incorporated.

The southwestern willow flycatcher *Empidonax trailii* subspecies *extimus* (sw WIFL), was placed on the Federal endangered species list in March 1995. This small, neotropical-migrant, mid-summer breeding, riparian obligate bird breeds in southern California, most of Arizona, eastward to the Rio Grande in New Mexico, in southwestern Colorado, in southern Utah and Nevada, and historically along the Rio Grande of westernmost Texas. Today, it does not occur east of the Rio Grande of central New Mexico or anywhere in Texas.

The interactions between the sw WIFL and its habitat was reviewed by Finch and Stoleson (2000), Sogge et al. (2003) and especially between it and saltcedar by DeLoach and Tracy (1997), DeLoach et al. (2000), and Dudley et al. (2000, 2005). Its populations have declined precipitously in recent years, in close correlation with the decline in its native willow-cottonwood riparian habitat and the increase of saltcedar. However, in mid-elevational areas of Arizona (but not in other states) it nests extensively in saltcedar in areas where saltcedar has replaced the native trees. It chooses saltcedar nest trees even if apparently suitable willows are abundant nearby. This appears to be a case of the classical ecological concept of a "super-normal stimulus" in which one stimulus (in this case the near ideal branching structure of saltcedar for nest placement) overrides all other stimuli even if such selection overall is detrimental to the bird. Nearly all known or suspected mortality factors of the sw WIFL are made worse by saltcedar, including loss of habitat, nest parasitism by cowbirds, need for free water in streams, lakes or flooded areas, lack of proper food (insect larvae), lethal high temperatures, wildfires, and possibly stress on the females from multiple nesting attempts after failures in saltcedar. This results in a reproductive success in saltcedar of only half that in cottonwood/willow dominated habitats (DeLoach and Tracy 1997, DeLoach et al. 2000, DeLoach et al., MS submitted 2000). However, substantial population increases recently have been reported as willows have revegetated, as along the middle Rio Grande of New Mexico and at Roosevelt Lake, Arizona.

A major concern stated by flycatcher biologists is that in many areas now occupied by saltcedar the water tables are too low and the soil salinity too high to allow revegetation by cottonwoods and willows after saltcedar control and the sw WIFL would loose its breeding habitat. This would be a concern only in Arizona because in other states the sw WIFL breeds only or mostly in native habitat. Also, in all the major sw WIFL breeding areas, both depth to water table and salinity levels are suitable for cottonwoods and willows, as evidenced by their presence; their low abundance is probably because of competition from saltcedar. Surveys by the Bureau of Reclamation (USDI-BOR 1995) demonstrated that along the lower Colorado River downstream from Lake Mead most of the potential breeding area is suitable for cottonwood/willow, including all of the major breeding area at Topock Marsh. The complete lack of breeding in this major area of former breeding along the Colorado River downstream from Topock Marsh is probably caused by the saltcedar invasion. Temperatures within the saltcedar thickets often exceed the lethal high temperature for survival of bird eggs, whereas the former upper canopy of tall cottonwoods and understory of willows was cooler. Several areas along the river have revegetated naturally with cottonwoods and willows since the El Niño floods of the mid 1980s and mid 1990s (DeLoach and Sarah Wynn, USDI-BOR, Denver; personal observations, 2001).

Major revegetation experiments are underway by the Bureau of Reclamation to develop methodologies for restoring the native vegetation. Large projects are in progress at San Marcial on the Rio Grande and are planned for Lake Meredith and Big Bend National Park, TX and along the Lower Colorado River, CA/AZ (Ken Lair and Sarah Wynn, BOR, Denver). At recent manual revegetation sites along the lower Colorado, the transplanted cottonwood and willow poles are growing beautifully and rapidly (DeLoach and Sarah Wynn, personal observations, 2001).

2. Depletion of Water Resources and Degradation of Water Quality.

Numerous large-scale experiments measured water usage by saltcedar from the 1940s to the 1980s, along the Gila River, NM (Gatewood et al. 1950; Culler et al. 1982), the middle Rio Grande (USDI Bureau of Reclamation 1972, 1973; van Hylckama 1968, 1974, 1980; Gay and Fritschen 1979), the lower Colorado near Blythe, CA (Gay and Samis 1977, Gay and Hartman 1982, Gay 1985), and along the Pecos River near Artesia, NM (Weeks et al. 1987). Usage was greatly influenced by depth to water table, water salinity, density and size of the plants, growth stage of the plant, season of the year (temperature/daylength), and latitude/elevation above sea level (also temperature/daylength). Summaries of this research by Johns (1989), Horton (1989) and DeLoach (1991) indicated that water usage by saltcedar varied from 3 ft/yr at Bernardo, NM to an average 5.7 ft/yr at Blythe, CA.

At Artesia, NM from 1980 to 1982, old growth saltcedar (10 ft water table) used 2.75 mm/day, wet old growth (2-3 ft water table) used 5.2 mm/day, burned in 1974 (4-6 ft water table) 3.65 mm/day, and mowed in 1977 (10 ft water table) used 4.87 mm/day. Average usage in all plots was 35.4 (30.1 to 42.1) inches/year and replacement vegetation (grass and forbs) used 22.4 to 26.4 in/year, giving a calculated salvage of 11.0 in/year by the energy-budget method or 7.9 in/yr by the eddy correlation method (Weeks et al. 1987).

Along the Rio Grande, one-third of the allowable annual depletion of water is lost to saltcedar (Steve Hansen, U.S. Bureau of Reclamation, Albuquerque, personal communication). Water used by saltcedar, above and beyond that used by the native vegetation, is estimated to be sufficient to supply the needs of 20 million people (Tim Carlson, *Tamarix* Coalition, Grand Junction, CO, personal communication). The present severe drought has reduced the streamflow available for irrigated agriculture and municipal use, threatening the livelihood of farmers, and causing water rationing in towns and cities. Flow from the Rio Grande no longer reaches the Gulf of Mexico. This has resulted in default of water agreements between states and between the United States and Mexico, with serious economic and political consequences. Reduction in flow seriously degrades water quality since pollutants are not flushed out and continue to accumulate. Large-scale and expensive saltcedar eradication programs have been initiated by the Departments of Agriculture of Texas and New Mexico, by many affected water districts, and as proposed for Federal funding in these and other western and southwestern states.

Some studies also showed that water usage by native phreatophytes, especially by cottonwoods and willows (the most valuable wildlife habitat) was equal to saltcedar (reviewed by DeLoach 1991). However, the studies did not consider that saltcedar, because it is a deep-rooted facultative phreatophyte, can utilize water from much deeper in the soil, and can occupy an area much further from the streambanks or lakeshores, and thus occupy a much larger area of the valley and can consume much more water on a river-valley basis than can willows and cottonwoods (Smith et al. 1998).

3. Causes of Saltcedar Invasion.

The invasion of saltcedar is thought by many to be caused mostly by abiotic or human-produced environmental changes, i.e. dam building, livestock grazing, groundwater pumping, etc. and that the invasion was passive and only followed these changes (Everitt 1998, but contradicted by DeLoach et al. 2000). Saltcedar's innate aggressive characteristics appear to make its invasion unstoppable and its domination of ecosystems to appear invincible. Saltcedar appears to be more aggressive and better adapted to the changed environment than are the native plant communities it has replaced. Saltcedar qualifies under 10 of the 12 criteria that Baker (1974) used to characterize the ideal weed.

However, saltcedar also has invaded small streams and desert springs far removed from altered river hydrologic cycles, livestock, or other obvious human influence (Lovich and deGouvenain 1998, Barrows 1998). Its invasion also is promoted by several important biotic factors that are little recognized by the proponents of the "passive invader" hypothesis: *i.e.* its direct competition with the native plants for water, nutrients, light (Smith et al. 1998); its synergistic interactions with the abiotic/anthropogenic factors; its alteration of the physical environment to its own competitive advantage (increased soil salinity and wildfires and decreased water availability); and very importantly, the lack of natural enemies (insects, plant pathogens) that damage it (DeLoach et al. 2000).

The unique ecological and physiological characteristics of saltcedars allow it to interact synergistically with many natural factors or human ecosystem modifications in a feed-forward manner to increase its own competitive advantage over the native plant communities. The construction of dams alters the natural flood cycle to exclude spring germination of cottonwood/willow seeds but to allow summer germination of saltcedar seeds, saltcedar lowers water tables below the root level of the native cottonwoods and willows, it increases wildfires and soil salinity to which it is tolerant but which kill the natives, it is more tolerant of livestock browsing than are the natives, and herbicide or mechanical controls used to control it also kill many native plants. Importantly, the native insects and plant pathogens constantly suppress native plant communities but they do not damage saltcedars (DeLoach et al. 2000). However, two saltcedar natural enemies (introduced several years ago by unknown means), a leafhopper (*Opsius stactogalus*) and a scale insect (*Chionapsis eutrusca*), which act as biological control insects, have a suppressing effect on saltcedar in several areas.

4. Conventional Control of Saltcedar.

Saltcedar, during the past 50 years, has proven to be a difficult and expensive invasive weed to control. They propagate both by huge numbers of tiny windblown or waterborne seeds and vegetatively, they are facultative phreatophytes and halophytes, and they are tolerant of fire, drought and inundation. Programs to control saltcedar (and native phreatophytes as well) have been conducted several times in the past, most notably during and after the drought of the 1950s (PSIAC 1966, Pinkney 1990, Sisneros 1990; reviewed by DeLoach 1989b, DeLoach and Tracy 1997), but the effect always has been short lived because of regrowth and reinvasion. The present drought makes rapid control urgent.

Large-scale herbicidal and mechanical control programs are in progress along the Pecos River of Texas and New Mexico and are planned to include the Rio Grande, and the Colorado, Brazos, Frio, and other infested rivers and their tributaries in western Texas. Similar programs may be initiated in several other western states. These treatments primarily use Arsenal and Rodeo applied by helicopter (Hart et al. 2000, Duncan and McDaniel 1998). In areas of present monotypic saltcedar stands (especially prevalent along the saline Pecos River) these controls are expected to provide rapid control and immediate water salvage, and with little or no detrimental side effects, though several years will be required to treat all areas. However, if herbicides are applied to areas of mixed saltcedar/native stands, severe damage will be done to the natives, especially to cottonwoods and willows which are the prime species for wildlife habitat.

5. Appropriateness of Biological Control for Saltcedar.

a. Biological Control of Weeds in General. Biological control is highly specific, killing only one or a few closely related plants. It is most useful in natural areas, rangelands and forests, where the ideal objective is to kill only the target weed and leave unharmed all the other plants, which is the opposite of the objective for herbicidal control in cultivated crops.

Three approaches to biological control are usually recognized. In "Conservation", the methodology is to develop techniques that conserve the natural enemies that control the target pest. Unfortunately, the native insects and plant pathogens of the U.S. cause little damage to saltcedar. Certain cultural controls such as adjusting dates of cultivation or pesticide applications in crops, or the use of domesticated animals (especially goats or sheep trained to eat weeds) may be of use in rangelands but are risky in natural areas. In "Augmentation", methods are developed for increasing the numbers of control agents, such as by mass rearing and release. This method requires natural enemies that can be manipulated and usually is prohibitively expensive. The "Classical" or "Introductory" approach for weed control is to introduce the highly host specific natural enemies (usually insects or plant pathogens) that suppress the weed's populations in its homeland. The philosophy, methodologies, and safety guidelines and regulations have been well developed especially since the late 1950s (Huffaker 1957, 1964, 1971; Harris and Zwölfer 1968, and as reviewed by DeLoach and Tracy 1997, DeLoach 1997, DeLoach and Carruthers, in press, and Spafford-Jacob and Briese 2003, DeLoach 2004a). Today, they offer highly accurate methods for determining the safety of candidate control agents, but less accurate methods for predicting degree of control after release. Historically, this approach has been by far the most often used and the most successful (Julian and Griffiths 1999, Nechols et al. 1995). The classical approach is relatively inexpensive, permanent, highly host specific, and environmentally compatible. The objective is not to eradicate the weed (which biological control has never done) but to reduce the abundance below the level where economic or ecological damage occurs.

Biological control kills the target weeds even in mixed stands without harming other plants, the control agents actively seek out the target weed even in areas of difficult access, and it provides permanent suppression of the target weed so that reinfestation does not occur (therefore, 100% control to eliminate weed reservoirs of reinfestation is unnecessary). It does not contain chemicals that pollute the environment, and it is relatively inexpensive because every plant in the infested area does not need treatment and repeated applications are unnecessary. During the history of biological control of weeds, no damage has been reported to non-target plants except for 8 cases of minor damage during the 1960s, most of them of short duration, that would not occur under present guidelines and regulations. All cases of non-target feeding, including that of the well-known seed-head fly that controls must thistle, were predicted in the pre-release testing. No case of a control agent changing its host range is known (McFadyen 1998, Marohasy 1996, Gassman and Louda 2001).

Disadvantages of biological control are that the control agents, once released, cannot be limited to certain areas, control may be somewhat slow, requiring a few years to achieve satisfactory control level in a given area and several years to spread to other areas unless redistributed manually. Suitable control agents sometimes cannot be found that have narrow host ranges and also provide control in all climatic zones or in all habitats. Sometimes, naturally occurring parasites and predators limit the effectiveness and too-frequent applications of herbicides can prevent the control agents from reaching controlling levels.

Classical biological control has been used against 133 weed species in 75 countries, and using 365 introduced control agents since 1865 (Julien and Griffiths 1998). Control agents have been released to control 40 exotic weed species in the continental United States and Canada since 1945, and against 25 exotic weed species in Hawaii since 1902. About one-third of these weeds have been successfully and permanently controlled, with great benefit to natural areas and to agriculture. Another third have been partially controlled and a third with little or no control; many of the latter have received little research effort or are new projects. The success rate has increased to ca. two-thirds in recent years with more concentrated research and the development of improved technologies. Greatest effectiveness often is obtained by introducing control agents that attack different parts of the weed, such as foliage feeders, seed feeders, stem or root borers, etc. (McEvoy and Coombs 1999). In the continental United States, successful control has been obtained of St. Johnswort, puncturevine, tansy ragwart, muskthistle, alligatorweed, waterhyacinth, waterlettuce, skeletonweed, field bindweed, leafy spurge, and purple loosestrife (Nechols et al. 1995, Rees et al. 1996). Several other projects appear to be nearing success, such as melaleuca, giant salvinia, hydrilla, Old World climbing fern, Brazilian pepper tree, yellow starthistle, houndstongue, toadflax, some knapweeds, and saltcedar. However, in most of these successful projects, biological control was the only control used and herbicidal or mechanical controls were unnecessary.

The protocol for the "introductory" approach is to 1) find and select the best of the highly host specific insects or plant pathogens that damage the weed (those that cannot complete their life cycle on other plants) within the weed's native distribution in other countries (Goeden 1983), 2) determine the control agent's biology, ecology and host range, 3) introduce them into quarantine in the United States for final host range and biological testing and to produce "clean" colonies free of predators, parasitoids or pathogens; 4) after obtaining the proper authorizations, to release them into the field; and 5) monitor the control obtained and the effects produced in the natural and agricultural ecosystems.

The methodologies of biological control of weeds, including host-range determination of the control agents, have been developed to a high state of reliability over many years (Huffaker 1957, Harley and Forno 1992, Rees et al. 1996, DeLoach 1997). A variety of tests are used depending on the life history of the control agent, such as adult or larval feeding, either no-choice or multiple-choice, or ovipositional host selection (Huffaker 1964, Harris and Zwölfer 1968, Zwölfer and Harris 1971). Plants for host specificity testing are selected by the centrifugal-phylogenetic method whereby plants most closely related to the target weed (same genus) are tested first; if feeding occurs on other species, then species of other genera (same family) are tested, and so on until the host range is defined or the test insect is shown to have too broad a host range to be introduced (Harris and Zwölfer 1968, Wapshere 1974). Since no species of the family Tamaricaceae are native or are beneficial exotics [except for the exotic athel (*Tamarix aphylla*)] in North America, a control insect would be acceptable for introduction so long as it does not complete its life cycle on species outside the Tamaricaceae and does not cause great damage to athel, and does not damage the native *Frankenia* spp. (Family Frankeniaceae).

b. Biological Control of Saltcedar. Saltcedar ranks very high under nearly all of the characteristics generally accepted as qualifiers for biological control: it is an exotic invader, it is not closely related to any native or economically important plants in North America, it causes great losses and has small beneficial values, it occurs in stable ecosystems, and many promising control agents are known in its native range that are highly specific and potentially could be introduced (DeLoach 1989a, 1991, DeLoach et al. 1996; DeLoach and Tracy 1997).

Biological control offers the potential for effective control of saltcedar. The insects introduced or proposed for introduction are highly specific to saltcedar and can control only it in mixed stands without damage to any other plants. Biological control is relatively inexpensive and provides permanent control, including control of regrowth and of reinfestations. Although it will not eradicate saltcedar (nor will any other type of control), the 75 to 85% control expected (which could reach 95% control in some areas) is sufficient to greatly reduce water losses; to allow recovery of native vegetation, wildlife, and fishes; to reduce wildfires and salinization of soils; and to allow satisfactory recreational usage of riparian areas. The potential for successful control is great based on the large number of host-specific insects known to attack saltcedar in the Old World and on early field test results with leaf beetle, *Diorhabda elongata.*

The major concern in the use of biological agents to control saltcedar is for the possible loss of habitat for the endangered southwestern willow flycatcher (sw WIFL) (*Empidonax trailii extimus*) that has begun nesting in saltcedar in mid-elevational areas of Arizona and southernmost Nevada in recent years, since its native willow nest trees have been replaced by saltcedar (DeLoach and Tracy 1997, DeLoach et al. 2000). This was the main topic addressed by the Biological Assessment submitted to the U.S. Fish and Wildlife Service in October 1997 (DeLoach and Tracy 1997) and of the Research Proposal of 28 October 1998 to FWS (DeLoach and Gould 1998). However, the Biological Assessment (and DeLoach et al. 2000) concluded that biological control is unlikely to adversely affect the sw WIFL or any other of the 51 endangered or threatened species that occur in or near saltcedar infested areas of the United States. In fact, biological control of saltcedar is expected to improve the status of most species since control of saltcedar is given as part of the recovery plan of many species (Anonymous 1995).

Biological control is not expected to adversely affect, and probably will be beneficial to any threatened and endangered species near saltcedar infected areas of Texas or eastern New Mexico – the Texas poppy mallow (*Callirhoe scabriuscula*), the Pecos puzzled sunflower (*Helianthus paradoxus*), the Concho River water snake (*Nerodia harteri paucimaculata*) or three fish species, the Leon Springs pupfish (*Cyprinodon bovines*), the Comanche Springs pupfish (*Cyprinodon elegans*) or the Pecos gambusia (*Gambusia nobilis*). Any possible adverse effects of biological control on the sw WIFL is not expected to be a factor in the Upper Colorado, TX saltcedar control project. The flycatcher does not and never has occurred within the control area, the nearest sw WIFL breeding area (only a few nests in saltcedar stands) are at Elephant Butte Lake State Park and at the Sevilleta NWR, on the middle Rio Grande, NM, more than 200 miles to the west, and with no streams that connect the Rio Grande and the Colorado River of Texas.

The principal disadvantages of biological control are 1) that 3 to 5 years probably would be required for it to achieve its potential in an area of a mile radius around a release site. However, control could be obtained throughout Sector 1 of the project, from Lake Thomas dam to the headwaters (if the beetles are as effective as indicated in recent field tests), if they are redistributed manually with release sites each ½ to 1 mile apart. Such releases are inexpensive once a large population of beetles is established at one location in the field and are available for redistribution. The degree of control that will be produced by the *D. elongata* beetles along the Colorado River is still somewhat uncertain. Both the physical and the biotic environmental factors vary between locations and their effect on the beetles cannot be fully predicted before release. 2) the insects will spread throughout area of climatic adaptation, possibly into areas where they are not wanted.

Biological control can be useful in saltcedar control programs in several different ways. Preliminary results at the better of our release sites indicate that a) the *D. elongata* beetles may provide satisfactory control of large, monotypic stands or of disperse stands mixed with native vegetation, and without other types of control, as has occurred with the majority of the past successful biological control of weeds programs. However the method used alone is moderately slow (3-5 years) and the effectiveness for saltcedar is not yet completely demonstrated. Biological control also can be used b) to follow herbicidal treatments to control regrowth and reintroductions of saltcedar, c) in areas of mixed native/saltcedar vegetation where protection of the native plants is important and where the hand application of herbicides that would be required to protect the native plants is prohibitively expensive, d) in areas where herbicides are unlikely to be used over the next 3 or 4 years, and e) to obtain long-term and permanent control. Once the initial dense saltcedar stands have been reduced by herbicides and the biological control insects have become established, further herbicidal control may be unnecessary. In fact, the continued frequent use of herbicides is likely to prevent permanent, effective biological control by reducing the food supply of the control insects so that they cannot maintain controlling populations to provide continuing control of regrowth and reinvasion.

6. Previous Research on Biological Control/Saltcedar

Biological control of saltcedar was begun by USDA-ARS at Albany, CA in the 1970s with explorations for candidate natural enemies in Israel, Italy, Turkey, Syria, Iran, India and Pakistan. This research and that of scientists in the Soviet Union, revealed over 300 insect species in Asia, with several also in southern Europe and northern Africa, that damage saltcedar but that apparently do not attack other plants. Research toward testing and release of natural enemies was begun by USDA-ARS at Temple, TX in 1987, joined by USDA-ARS at Albany, CA in 1998. Some 20 species are undergoing preliminary testing by overseas cooperators in Kazakhstan, China, Israel and France and some 10 species are being tested in quarantine at Temple and Albany (DeLoach 1989b; DeLoach et al. 1996). Three species have received TAG recommendation for field release, the leaf beetle *Dirohabda elongata* from China and Kazakhstan, a mealybug *Trabutina mannipara* from Israel, and a foliage-feeding weevil *Coniatus tamarisci* from France.

a. *Diorhabda elongata* (leaf beetle) from Fukang, China and Chilik, Kazakhstan.

The *Diorhabda elongata* beetles (Fig. A5-3, page 12a) have good potential for highly effective, safe and cost-efficient control of saltcedar.

(1) Host specificity and safety. The subspecies *D.e. deserticola* from Fukang, China and Chilik, Kazakhstan has been extensively tested at Temple since 1992 and also at Albany since 1999. Its ability to develop, reproduce and complete its entire life cycle has been tested on 84 test plant accessions, including 6 species and 22 accessions of *Tamarix*, 4 species of the somewhat related and native *Frankenia*, and 52 species of more distantly related plants, habitat associates, agricultural crops, and ornamental plants (DeLoach et al., 2003; Lewis et al. 2003a; Milbrath and DeLoach, a,b, manuscripts in review).

These tests, and summaries by DeLoach 2004a, b and DeLoach et al. (in press a, b), demonstrate conclusively that *D. e. deserticola* can feed as larvae or adults, is attracted to and lays eggs on, or completes its entire life cycle only on species of two plant genera - *Tamarix* and *Frankenia*. However, development, attractance to, and oviposition on *Frankenia* in cages was so low that completion of its life cycle on these plants is rare, and they are not expected to sustain a population on these plants in the field, except possibly on *F. salina* which grows from California to Chile. Development and reproduction on the distinctive, exotic, large, evergreen tree, athel (*Tamarix aphylla*), that is a shade tree of some beneficial value in southwestern desert areas, especially in northern Mexico, was only 20 to 25% of that on the target saltcedars. The beetle is expected to feed on and colonize athel to a minor extent after release, but not to cause important damage to the trees (Table Append. A-1).

Table Append. A-1. Multiple-choice host selection test by larval and adult *D.e. deserticola* from Fukang, China and Chilik, Kazakhstan, 2000, at Temple, TX[a]

	Mean % on each test plant during test, normalized to 100% of total (number of replications)		
Test plant	Larval survival[b] egg to adult	Adults on plants[c]	Eggs laid on plants[c]
T. ramosissima (WY)	29.3 (13)	43.8 (29)	45.7 (35)
T. parviflora (CA)	13.0 (24)	28.7 (4)	33.7 (7)
T. aphylla (TX)	18.0 (15)	27.0 (17)	19.7 (20)
F. jamesii (CO)	6.7 (12)	0.25 (32)	0.93 (35)
F. salina (CA)	12.4 (23)	0.19 (32)	0.00 (35)
F. johnstonii (TX)	4.3 (10)	0.06 (32)	0.00 (35)
F. palmeri (CA)	16.2 (7)	-	-
Total counted: all reps		1,596	8,846

[a] Data is from Lewis et al. (2003a) and DeLoach et al. (2003).

[b] No-choice tests in sleeve bags on growing, potted test plants outdoors at Temple, TX or in the greenhouse at Albany, CA.

[c] Multiple-choice tests in 3X3X2(h) m outdoor cages (5 tests, 29 reps), small outdoor cages (1 test, 3 reps), (Fukang beetles); or greenhouse in 1.4X1.5X0.5 (h) m cage (Chilik beetles only, only eggs counted, 1 test 3 reps).

Diorhabda elongata deserticola from Fukang and Chilik has received U.S. Fish and Wildlife Service concurrence, all NEPA clearances, and USDA-APHIS-PPQ permits for release. It was released into field cages during the summers of 1999 and 2000 at 10 sites in Texas, Colorado, Wyoming, Utah, Nevada and California. It successfully overwintered and heavily damaged saltcedar in cages at six of these sites: Pueblo, CO; Lovell, WY; Delta, UT; Lovelock and Schurz, NV; and Bishop, CA. The beetles did not overwinter at the Seymour, TX site, but those added to the cages in the spring heavily damaged the plants during the summer. The beetles were released from the field cages and into the open field in May 2001 at all 6 sites where they overwintered (Pueblo, Lovell, Delta, Lovelock, Schurz, Bishop all north of the 37th parallel), and at Seymour. Beetle populations developed in the surrounding saltcedar plants at Pueblo, Lovell, Delta, Lovelock and Schurz (DeLoach et al. 2004) but not at Seymour which is south of the 37th parallel (Fig. A5-4).

(2) Field Establishment and Control. At Lovelock, NV (so far the best site) the Fukang/Chilik beetles established and reproduced readily in the field. By August 2002, they had increased to over 100,000 and had completely defoliated all saltcedar over a 2-acre area and numerous adults and larvae were present in an area twice this size. By July 2003, the first generation adults and larvae had defoliated an area of ca. 8 acres. After the third growing season, in September 2003, the beetles had increased to several millions and completely defoliated from 70 to 500 acres of saltcedar at Lovelock (Fig. A5-5) and Schurz (Fig. A5-6), Delta, Pueblo, and Lovell (DeLoach et al. 2003). By September 2004, an area of from 3000 to 10,000 areas had been defoliated at Lovelock (Fig. A5-6), extending for 11 miles along the Humboldt River, and beetles had been found along 100 miles of the river.

At Schurz and Delta, defoliation totaled 300 to 1000 acres each and for a distance of ca. 2-3 miles along the Walker and Sevier Rivers, at Pueblo ca. 300 acres, and at Lovell ca. 200 acres. Sufficient numbers of beetles were produced at Lovelock in both 2003 and 2004 to release 1400 adults at the intersection of 0.5 mile grids throughout all the saltcedar infested area in the western United States. If the beetles are as successful as at Lovelock, all saltcedar in the United Stats could be defoliated within 3 or 4 years.

In our previous releases in field cages, severe defoliation during 2 years killed even medium-sized trees. During 2003 and 2004 in the open field, many saltcedar plants had resprouted from the base, and some had resprouted from upper branches but most of the upper stems had died. During each generation, adults and larvae killed most of this regrowth. We expect that spring growth during the fifth growing season (2005) will reveal that many small to medium-sized plants have been killed and most other plants have been reduced to 10% or less of their former size. At some locations during 2002, predation seriously reduced the effectiveness of the beetles during the first or second years after release. However, the beetle populations at Lovell and

Delta recovered and during next year went on to produce severe defoliation over many acres. The response during the second year at Lake Thomas, Artesia and Kingsville is not yet known. At all of the 5 established sites north of the 37th parallel and within the area of beetle attack, the defoliation of saltcedar appeared to exceed 95% during 2003 and 2004. However, it is still too soon to know if this level can be maintained or how many plants are completely killed. DeLoach and Gould (1997) estimated that 75 to 85% control in natural areas was sufficient to prevent damage to natural ecosystems and to improve water conservation.

(3) Failure of Fukang/Chilik Beetles in Texas and Other Areas South of the 37th Parallel. The *D. e. deserticola* beetles originally from Fukang, China (latitude 44°4'N) and Chilik, Kazakhstan (latitude 43°33'N) did not overwinter in cages at Seymour, Dallas or Temple, TX nor at Hunter-Liggett Military Base, CA, nor after release into the open field at Seymour, TX or Bishop, CA. Beetles placed in field cages in the spring at the Texas locations developed normally and produced another generation of adults by late June. However, this generation did not oviposit, ceased feeding, entered diapause in mid-July, and died during the winter. Observations indicated that the probable cause was that the summer daylength at these most southern sites is too short to prevent diapause. The beetles then starved during the 8 months before saltcedar foliage became available in March (Lewis et al. 2003b). These observations were confirmed by our collaborator (Dan Bean) at Albany, CA, who demonstrated in intensive laboratory studies that *D. e. deserticola* requires a minimum of 14 hr. 45 min. to prevent the initiation of overwintering diapause; maximum daylength at Seymour (33.3°N) at the summer solstice is only 14 hr. 21 min., is somewhat less at Dallas, and is 14 hr 10 min at Temple (31.1°N). We conclude that these beetles will not control saltcedar in Texas nor in other locations south of ca. 37°N latitude (the northern border of OK, NM and AZ) (Lewis et al. 2003b; Dan Bean, USDA-ARS, Albany, CA, manuscript in review).

b. Potential of other *Diorhabda elongata* biotypes to control saltcedar in Texas and south of the 37th parallel.

During 2002 and 2003, we received shipments into quarantine of 4 additional biotypes of *D. elongata* (different from the Fukang/Chilik biotype), from Turpan, China; Crete, Greece; Sfax, Tunisia and Karshi, Uzbekistan. In laboratory tests at Albany, CA all 4 of the new biotypes appeared to be adapted to short daylengths south of the 37th parallel. During the summer of 2003 and 2004, we released these beetles into field cages at 8 locations and the Crete beetles were released into the open field at 7 locations (see paragraph 2c, Table 5, below), to determine which biotype overwinters, develops best, and damages saltcedar the most there. Then, the best biotype will be released into the open field at other locations during 2005. The releases at Lake Thomas and Big Spring are part of the Texas Colorado River Saltcedar Control Project, in which the entire area from Lake Thomas dam to the headwaters (Segment 1) is set aside for biological control, and section 3 is to receive biological control during the third year following herbicidal treatments (McGinty and Thornton 2003).

(1) Host Specificity and Safety. The Crete beetles were collected along the north shore of Crete, at 35°20'N latitude, or similar to that of Amarillo, TX. These appear slightly

different morphologically from *D. e. deserticola* and may be a different species. During the summer of 2002, we conducted the full spectrum of host range tests with these Crete beetles as done previously with the Fukang beetles. The host range seemed to be identical to the Fukang beetles previously released except for slightly more development and oviposition on athel and *Frankenia salina*. We also tested *D. e. deserticola* (the same subspecies as the Fukang beetles) but collected near Turpan, China only 100 miles southeast of Fukang. These beetles appear to be identical to the Fukang beetles in every way except for daylength response.

More recent tests, conducted from June to August 2003 (Milbrath and DeLoach, MS accepted), compared *D. elongata* beetles from Crete, Tunisia, Uzbekistan and Turpan. No-choice tests of neonate larvae on several agricultural and distantly related plants, and habitat associates demonstrated that the larvae did not feed on these plants, none developed beyond the 1st instar, and none produced adults (Table Append. A-2).

Table Append. A-2. Percent survival from neonate larvae to adult of four *Diorhabda elongata* biotypes on agricultural plants and habitat associates: no-choice test in sleeve bags, Temple, TX, June 2003[a].

Test plant	% Survival of beetles of different origins (Latitude)			
	Crete, Greece (35°20'N)	Sfax, Tunisia (34°46'N)	Karsi, Uzbekistan (39°55'N)	Turpan, China (42°57'N)
Tamarix ramosissima	63	94	69	88
15 Agricultural plants	0	0	0	0
T. ramosissima		94	69	46
13 related or habitat associates	0	0	0	0

[a] From Milbrath and DeLoach a (manuscript accepted).

Adults were tested individually but at the same time in paired choice tests (1 saltcedar and 1 *Frankenia* plant together) in small cages outdoors at Temple. These beetles were strongly attracted to saltcedar but not to *Frankenia* and they laid 365 to 545 eggs on *T. ramosissima*, 0 to 5 on *Frankenia*, and 22 to 71 on the cage walls (Table Append. A-3).

Table Append. A-3. Ovipositional host selection by female *Diorhabda elongata*: Paired-choice adult tests, Temple, TX, July – August 2003[a]

	Total no. eggs per plant during 5 days			
Location of eggs	Crete, Greece	Sfax Tunisia	Karshi, Uzbekistan	Turpan, China
Test 1		*T. ramosissima* vs. *F. jamesii*		
Tamarix ramosissima (CO)	**533**	**498**	**545**	**464**
Frankenia jamesii (CO)	**0**	**4**	**0**	**0**
Cage walls	29	22	52	24
Test 2		*T. ramosissima* vs. *F. johnstonii*		
Tamarix ramosissima (CO)	**446**	**465**	**406**	**365**
Frankenia johnstonii (TX)	**0**	**4**	**5**	**0**
Cage walls	36	34	71	62

[a]Outdoor tests in screen cages 56X67X122 (ht) cm, each cage with 20 beetles (10 males, 10 females) and 2 plants (1 *Tamarix* and 1 *Frankenia*), 5 replications (cages) of each test/beetle type. Data from Milbrath and DeLoach a, manuscript accepted).

In multiple-choice tests (3 or 4 saltcedar, athel, and 2 or 3 *Frankenia* plants together in the same cage) in large cages outdoors at Temple, adults from Crete, Tunisia and Fukang laid 16 to 42% of their eggs on each of the saltcedar plants, but only 7 to 9% on athel and none on *Frankenia*. The Uzbekistan beetles laid more eggs on athel and fewer on *T. canariensis* (Table Append. A-4).

Table Append. A-4. Ovipositional host selection of *Diorhabda* beetles: multiple-choice test in large (3X3X2(h)m) cages, outdoors, Temple, TX, 2002-2003[a].

	% Total eggs laid during 4 days and origin of beetles			
Test plant	Crete (2002)	Tunisia (2003)	Uzbekistan (2003)	Fukang, China (2002)
T. ramosissima	28	18	27	42
T. parviflora		33	17	
T. chinensis	33			21
T. chinensis X *canariensis*		26	22	
T. canariensis	31	16	7	22
T. aphylla	7	7	19	9
Frankenia salina	0	0	1	0
F. johnstonii	0	0	0	0
F. jamesii	0			0
On cage	0	0	4	5
Total	99	100	97	99

[a]Data from Milbrath and DeLoach a (manuscript accepted).

(2) Climatic adaptation of the new *Diorhabda* biotypes. The origin of the new biotypes of *Diorhabda* obtained from Crete and Posidi, Greece; Tunisia; and Uzbekistan; mostly are from latitudes much further south (34°46′ to 39°58′) (the Turpan, China beetles from 42°57′) than the previously released beetles from Fukang, China (44°10′) and Chilik, Kazakhstan (43°33′N) that were released in Nevada, Utah, Colorado and Wyoming. Laboratory studies by our collegue (Dr. Dan Bean) at Albany, CA indicated that the Crete, Posidi, Tunisia, and Uzbekistan beetles are capable of avoiding premature overwintering diapause and are adapted to the short summer daylengths in more southern areas of Texas, New Mexico and southern California and the Turpan beetles to intermediate areas somewhat further north (Bean et al., MS in press).

The Crete, Tunisia and Uzbekistan beetles have successfully overwintered in outdoor cages in Texas (Table A5-1, page 16) and the Crete beetles are established in the open field at Big Spring, TX and Artesia, NM.

(3) Release in Field Cages and in the Open Field. A Letter of Concurrence from FWS was issued on 13 June 2003 and Release Permits from APHIS were issued on 2 July for release at all requested sites in Texas including Seymour, Meredith Lake, Lake Thomas/Beal's Creek, Candelaria, Zapata, and San Jacinto. Turpan beetles placed in a field cage at Seymour, TX in March 2003 increased slowly at first but during July increased rapidly and severely defoliated the saltcedar. These beetles were released into the open field at Seymour on 30 July and placed in cages at Lake Thomas on 31 July. The Crete beetles were placed in field cages at Seymour, Lake Thomas, and at Beal's Creek on 8 July (Table A5-1, page 16).

(4) Projected effectiveness. The previously released *Diorhabda* beetles from Fukang, China and Chilik, Kazakhstan are rapidly defoliating saltcedar at 5 sites north of the 38th parallel in Nevada, Utah, Colorado and Wyoming. A good first estimate of numbers of plants completely killed will be available in the spring of 2005, but at present, most plants still are resprouting but are again defoliated by each generation of beetles. In these northern areas, the *Diorhabda* beetles complete 2 generations during the year, with major defoliation by mature larvae of the 1st generation in late June and a much larger defoliation by mature larvae of the 2nd generation in late August. The attack by the beetles covers most of the growing season of saltcedar, with the greatest damage during the later part of the season. These beetles did not overwinter or establish south of the 38th parallel.

The behavior in the open field of the Crete beetles released near Big Spring, TX (Lake Thomas, Beal's Creek and Buzzard Draw) is presently under study. However, the Crete beetles overwintered at Temple with very low losses and increased rapidly in cages during the spring of 2003. The Turpan beetles have not yet overwintered in field cages but laboratory tests project that they should. In field cages at Seymour, the Turpan beetles increased to high populations during July, and laid many eggs, a month later than had the Fukang beetles there during 2000, which is an additional generation more than the Fukang beetles (the Fukang adults did not lay eggs after June). These experiments indicate that the Crete beetles can establish and can control saltcedar in the climatic/daylength zones of Texas, unless suppressed by naturally occurring

biotic agents. The ability of the Turpan beetles to overwinter in the southern areas is questionable.

Damage by the Crete, Tunisian, and Uzbekistan biotypes in the southern areas is expected to be similar to that of the Fukang/Chilik beetles in the northern areas. However, in these southern areas, the beetles are expected to complete 4 or 5 generations, which will cover the longer saltcedar growing season in the south. This many generations would allow for a greater beetle population during the season and perhaps greater damage to saltcedar and greater dispersal of the beetles.

DeLoach and Tracy (1997) suggested that 75 to 85% control of saltcedar would prevent measurable environmental damage to plant and animal communities and would conserve substantial amounts of water. Preliminary observations of both defoliation in the northern areas suggests that control could exceed that amount and could reach 95 to 98% in some areas if plant kill or size reduction parallels the amount of defoliation observed.

c. Monitoring.

Monitoring of the *Diorhabda* beetles, their effect on saltcedar, and their possible attack on non-target plants was required by the Letter of Concurrence from FWS and by the APHIS release permits. This was carried out in field cages for 2 years before release in the open field and is continuing at all sites after release into the open environment. FWS also required monitoring of the recovery of the native plant communities and of the recovery of wildlife communities after biological control. Pre-release monitoring of native plant and native bird communities have been conducted at Seymour (3 years, see Fig. B1-5, page 32e), Lake Thomas and Big Spring (1-2 years) and are continuing into the post-release phase.

d. Clearances.

In March 1994, we submitted a second petition to TAG-PPQ-APHIS asking their recommendation for release of *Diorhabda elongata* into the open field. However, the listing of the southwestern willow flycatcher as federally endangered in March 1995 required consultation with the USDI Fish and Wildlife Service (FWS). We submitted a Biological Assessment, to FWS Region 2, Albuquerque, NM in October 1997. On 28 August 1998 we submitted a Research Proposal to FWS for release of the *Diorhabda* beetles. This document included a research phase in which; 1) *D. elongata* could be released only into secure field cages at 10 specified sites in different climatic zones in Texas, Colorado, Wyoming, Utah, Nevada and California, all more than 200 mi from where the southwestern willow flycatcher nested in saltcedar. The beetles would be monitored in the cages for one year to determine their survival, developmental biology, rate of increase, and observed damage to saltcedar and non-target plants in the cages. 2) The beetles then were to be released into the open field for a 2-year period, during which the degree and rapidity of control, rate of natural dispersal, and effects on native plant and wildlife communities would be monitored. After this combined 3-year research period, FWS and APHIS would review the research results and determine the conditions under which an

Implementation Phase could be carried out in which unlimited releases could be made in specified areas. A Letter of Concurrence was issued by FWS on 28 December 1998 (revised 3 June 1999), an Environmental Assessment was published by APHIS 18 March 1999, a Finding of No Significant Impact (FONSI) was issued on 7 July, and APHIS permits to release in the field cages were issued during July 1999; all contained these restrictions. We received a Letter of Concurrence from the U.S. Fish and Wildlife Service (Mr. Renne Loehoefener, 1 September 2004) allowing releases of *Diorhabda* beetles anywhere within the state of Texas, and a similar authorization from the Texas Department of Agriculture (Dr. Awinash Bhatkar) in September 2004.

APPENDIX B

A. Standard Operating Procedure (SOP)

1. Selection Criteria for Release sites for *Diorhabda* beetles. Release sites for *Diorhabda* beetles will be selected according to the following criteria:

a. In areas south of the 37th parallel. In this area, which is the northern border of Oklahoma, New Mexico and Arizona, the ecotype from Crete and Posidi, Greece appears to be the optimal ecotype for release. This is based on our test results to date among the ecotypes from (1) Fukang, China/Chilik, Kazakhstan; (2) Tunisia, (3) Uzbekistan, or (4) Turpan, China that either have been released or have been tested in the laboratory in the U.S. The Crete ecotype appears to be well adapted to the short summer daylength south of the 37th parallel (where the Fukang/Chilik ecotype did not establish), it is somewhat more host specific to saltcedar in tests including *Frankenia* and athel, and it established more readily in field cages in some southern areas.

b. In areas 200 miles from willow flycatchers. Release is permitted only in areas more than 200 miles from where the southwestern willow flycatcher nests to an important degree in saltcedar. So far, we have FWS concurrence for release only north of the 37th parallel or in areas along the Pecos River or further eastward, including all of Texas, and in central California. Concurrence is being sought for additional release sites along the Rio Grande, NM and in southern California.

c. In areas where athel (*Tamarix aphylla*) is not of important economic value. Athel is killed by hard freezes so survives only in the southernmost areas of the U.S. (approximately where citrus can survive). It is of low value in the southern areas of CA, AZ, NM and TX but is of somewhat more value for shade trees and windbreaks in northern Mexico. We are withholding releases in the Rio Grande valley between Texas and Mexico until agreement to release is obtained from Mexican authorities.

d. Areas with low flood frequency. All *Diorhabda elongata* ecotypes in the Old World, so far as we know, pupate and the adults overwinter under litter on the soil surface. Flooding during these developmental stages probably will cause mortality by drowning.

e. Protection from fire, herbicidal or other saltcedar control practices. Release sites should be protected from other saltcedar practices for a distance of at least 1 to 3 miles and for a period of at least 5 years. This will allow time for the beetles to increase to high enough populations to allow dispersal to other areas. Evidence from the establishment failure at Artesia, NM in 2004 indicates that saltcedar control near the release sites concentrates native insect predators into the release site, which then can destroy the *Diorhabda* population.

f. Protection from public access. Initial release sites should be in areas of limited public access to avoid disturbance of the release cages, interference with data collection,

disturbance of the habitat, or removal of the beetles until they are well established and dispersing.

g. Access by research personnel. Research personnel must have landowner permission and access to the release site and to the surrounding area as needed to release the beetles, to collect data on establishment and dispersal of the beetles; vegetation, and wildlife and weather/soil water monitoring; and for establishment and fencing of release cages and weather stations. The landowner also should be encouraged not to reveal the location of the release site or to allow access to the public or to the news media for a period of 5 years unless authorized by the agency conducting the research (ARS, TAES or other).

h. Landowner/land manager agreements. Records of these agreements concerning research at and access to release sites will be recorded on Form C-25 and kept on file at USDA-ARS-GSWRL for a period of at least 5 years.

2. Log of field activities. All activities of the releases and all following monitoring activities will be recorded on the Field Activities and Monitoring Log (Form C-1). This record will include the site name, date of activity, and type of activity performed (control agent releases, all types of monitoring, public field demonstrations, control agent collections and redistributions, instrument maintenance, downloading of data, etc.). This log will serve as the overall record for all field activities and also as a checklist for activities coming due.

3. Placement of control agents into field cages or release into the open environment. The precise release point and all control agent releases will be recorded on Form C-2 and kept on file at the USDA-ARS-GSWRL, Temple for a period of at least 5 years. Also, USDA-APHIS Form 526 will be completed and forwarded to the appropriate officials at USDA-APHIS and to the State Department of Agriculture in the state where the releases are made.

4. Plot Layout

a. Begin from original point of open-field release, or from the original 2 marked defoliated trees if different; at the David Higgins Ranch, ca. 10 m W of the nursery cage.

b. Lay out 100 m transects each 22.5° through the saltcedar stand (i.e. NNE, NE, NEE, E, SSE). Place a red surveyor flag each ca. 10 m along each transect.

c. Locate 4 X 4 m quadrats along and near each transect. During the overwintering and 1st adult beetle generation (May through early July), locate quadrats ca. 10 m apart for the 1st 50 m so as to include some saltcedar trees; grassy areas between saltcedar patches do not need quadrats. Mark corners of quadrats with iron stakes driven to ground level (to prevent injury to livestock hooves, and that can be located with a metal detector) and red surveyors flags. Draw a map of the canopy cover and calculate m^2 canopy of each quadrat and/or measure canopy cover of each quadrat from high resolution aerial photographs. Plot transects and quadrats on digital aerial photographs of the study area and recorded on Form C-15.

d. As the beetle population increases and disperses, increase the length of the transect to stay ahead of the increasing beetle wave front, with quadrats spaced to measure beetle populations of each expanding wave of each generation; each wave is expected to be broader than the preceding wave and quadrats may be spaced further apart in each wave.

5. Weekly sampling of beetle, predator and competitor insect (i.e. leafhopper) populations, and saltcedar condition.

a. Sample unit. The basic sampling unit is the quadrat, and ultimately is numbers of insects or percent defoliation per square meter of canopy.

b. Selection of sample branches. In each quadrat, select 1-m long terminal branches, distributed uniformly within the foliage of the quadrat, 3 branches in the top one-third of the trees, 3 in the middle third, and 3 in the lower third, a total of 9 branches per quadrat. Permanently mark and label each branch (i.e. T#1, T#2, T#3, M#1, L#1 … L#3) and mark the 1 m distance from the terminal. The measured 1 m will move outward along the branch each week as the branch grows. Record on Form C-2, 3, 5, 8, 9, 10.

c. Sampling control insects. Each week, using stepladders as needed, count all adults, eggs and each instar of beetle larva on each sample branch and record on the record sheet (Form C-2).

d. Probability distribution. During the growing season, determine probability distribution of all *Diorhabda* beetles in each of the 3 levels of trees with low or high beetle populations and at different stages of defoliation. Record on Form C-5.

e. Sampling other insects and arthropods. Each week, after emergence of overwintering adults in the last of March, count all adults and larvae or nymphs of each species of predator (ladybird beetles, assassin bugs, ants, other) identified in situ by the ARS technicians and record on Form C-2. All insects will be left in place on the branches except that 1 or 2 sample unknown insects will be placed in a vial of alcohol for identification at the Temple ARS lab.

f. Parasitism of *Diorhabda* beetles. Samples of 10 of each stage (egg, 1st, 2nd, 3rd, and adult) of *Diorhabda* beetles from 3 quadrats with moderate to high beetle populations will be collected at each count date, returned to the Temple laboratory, and held in clear plastic boxes with saltcedar foliage until they reach the next stage (eggs to 1st instar larvae; larvae to adult), when the number of parasite will be recorded (Form C-6).

g. Assessment of plant conditions. Make a visual estimate of percent condition of each sample branch at the same time the insects are counted (i.e. - % green, % yellowed, % yellowed by leafhoppers, % defoliated by *Diorhabda*, % dead stems, dead plants, and for the entire quadrat. Record on record sheet (Form C-3).

6. Measurement of beetle dispersal.

a. Weekly observations along extended transects. On each sampling date, walk the transects beyond the last quadrat and note presence or absence of *Diorhabda* large larvae or adults until the limit of dispersal is located. Each 10 to 30 m, in the zone where present, count of all adults and larvae seen in 2 min. on each of 1-3 trees at ca. eye level. Record numbers of beetles and distance observed along transects on Form C-4. If 5 or more larvae/adults are observed per 2-min. count, establish new quadrats out to that point.

b. *Diorhabda* attractant pheromone. When a suitable attractant pheromone is developed for the Crete ecotype, establish pheromone traps outward along the transects and after 2 days record the number of *Diorhabda* adults caught (yellow sticky traps with a vial of attractant, suspended from a saltcedar tree branch). This should be done at or near the peak of each adult *Diorhabda* generation, or more often if time permits.

c. Remote sensing – aerial observations and photography (see paragraph 7.g., below). Remote sensing detects only saltcedar plants damaged or defoliated by the *Diorhabda* beetles and so it always trails actual beetle dispersal. However, it is a good tool as a starting point for measuring *Diorhabda* beetle dispersal, especially over wide areas and for detection of new satellite infestations when the beetles are dispersing rapidly, and in areas difficult to access from the ground.

We will use medium altitude aerial photography, from 2,500 to 5,000 ft, made once or twice annually by ARS, Weslaco, TX to determine the limits of saltcedar damage/defoliation, from where to begin ground measurements as in paragraph 6.a and b, above (Form C-15).

7. Measurement of saltcedar defoliation and control by *Diorhabda* beetles. After defoliation of each generation of 3rd instar *Diorhabda* beetle larvae (ca. June 15, July 15, August 15 and September 15), measure foliage conditions and degree of defoliation:

a. Visual estimate. Make a visual assessment of percent damage to each branch as in paragraph 5.d. above (the weekly count may be used), and also of the overall appearance of the quadrat, and record on Form C-3, 13.

b. Photography of 1-m terminal branches. The branches will be selected as described for "Sampling Beetle Density", above, and will use the same branches used for the beetle counts. Each branch will be photographed in color on each count date using a 4.1 megapixel (or better) digital camera, with a white background card held behind and against the branch that is graduated in 5 cm grid lines. The photographs will be made from the same distance, and so are to the same scale. The background card will bear the transect #, quadrat #, tree #, branch #, date and time, which will appear in each photograph (Form C-10).

The digital images will be color-separated using specialized software and the area of green and brown foliage measured. Reduction (or increase) in foliage from the previous week's measurement equals the amount of defoliation or regrowth (C-13).

Complications of this method are that a) some overlapping of foliage occurs so foliage area is underestimated to varying degrees. A set of reference photos will be prepared for use in correcting the measured green area of the sample branches (the brown foliage usually is so sparse that little overlapping occurs). b) Foliage yellowing through natural senescence or from attack by *Opsius* leafhoppers or *Chianapsis* can be measured by color separation but death or missing foliage probably cannot.

c. Direct measure by leaf area meter. Since this is destructive sampling, branches for measurement will be cut from saltcedar trees adjacent to but not included in the quadrats. This technique is very time consuming and is used as a standard for calibration of the other methods, not for periodic sampling of all quadrats. Before removing the branches, each will be measured and evaluated by the other methods (visual and photography). Twice a year, in May-June and again August-September, we will remove one 1-m long branch from 4 sample trees, 3 branches in each tree, 1 in the upper third, 1 in the middle third, and 1 in the lower third of the trees. These are held in large plastic bags for transit to the Temple lab, held in a cold room (ca. 40°F), and measured the following week (record on Form C-13).

In the laboratory, twigs 2mm diameter or smaller (with attached leaf bracts) will be removed, plus all foliage attached to larger stems. The foliage will be separated as green, yellow and brown (dead) and the leaf area of each type measured separately, and the remaining bare stems also measured (living and dead stems measured separately). All measured foliage and stems will be recorded separately on Form C-13.

d. Biomass measurement. After measurement on the leaf-area meter, the foliage and stems will be placed separately by category for each branch in small paper bags. These bags will be placed in a drying oven at Temple for 1 week, or until dry, then weighed (green, yellow, brown foliage and living and dead stems all separately) and recorded on Form C-13.

e. Light intercept. A Licor 1-meter long light bar will be used to measure light intercept of each entire sample plant in the field. The light bar is inserted into the lower third of the growing plant at 10-20 locations and the average reading recorded, compared with an open-sky reading beside the plant at the same time. Readings are made between 10:00 am and 3:00 pm on sunny days (Form C-11).

f. Foliage temperature. Temperature of green and defoliated trees will be measured with an Infrared Temperature and Humidity meter, Extech Model RH101. Several readings will be made from different directions of each quadrat, and the average reading recorded on Form C-12.

g. Remote sensing. The site will be photographed by low-level aircraft using 9 inch aerial film, flown at 500 ft altitude above the ground to provide very high resolution. This will be done in mid-to late September to capture nearly all defoliation occurring during the year (Form C-15). Computer software analysis of the images can provide an accurate measure of saltcedar canopy cover. This will provide a calibration of the above defoliation estimates and of the entire model in predicting the rate of dispersal (defoliation) produced by *Diorhabda* (see Section B2.C.5 Remote sensing).

h. Census of dead trees. A census of dead small, medium and large saltcedar trees will be made annually during the spring, after the living trees have leafed out. Trees with no green foliage will be examined by scraping or cutting the bark near the base of stems to determine if the cambium layer is living (green, wet) or dry (brown, dry). This information will be correlated with the number of years the trees have been defoliated as recorded in the periodic examination of plant foliage condition in Section B.2.C (Form C-3) and by remote sensing (Form C-15). Dead trees will be sampled separately each year to determine the percent of trees killed after each year of defoliation and thus the number of years of defoliation needed to kill small, medium and large trees.

(1) In established quadrats. All trees whose trunks originate in the established quadrats will be examined.

(2) Along transects. Additional transects will be established in defoliated areas outside the main sampling area. Trees will be sampled whose trunks originate in a belt 2 m on each side of a line transect, and recorded as distance along the transect.

8. Weathering monitoring. A central weather monitoring station will be located at Big Spring and at another site to continuously monitor temperature, humidity, rainfall, wind speed and direction and solar radiation. Four leads monitor soil and surface temperature under litter out to 100 ft from the station. Additional small HOBO monitors record temperature/RH within the trees. We will download the data ca. monthly to a notebook computer and perform analyses and graphs back at the laboratory and record on Form C-14.

9. Extrapolation of *Diorhabda* population and saltcedar damage to 1-m^2 basis for use in the model. We will extrapolate from 1-m branch counts, to quadrat (m^2) basis, to landscape basis by the following steps (Form C-17). All steps will be recorded separately for branches in the bottom third (to 1 ½ m high), middle third (1 ½ to 3 m high), and upper part (3 m to top) of the trees.

a. In the field (Form C-9) or laboratory, Form C-13, measure total length of foliage-bearing twigs per 1-m branch.

b. In the laboratory, measure total leaf area per 1-m branch and establish the ratio of leaf area to twig length (Form C-13).

c. Count the total number of 1-m branches per quadrat, and per 1 m^2 of saltcedar canopy in the quadrat (Form C-8).

Multiplying top, middle and bottom means per branch X number branches in each level, add the 3 levels, and divide by m^2 canopy in the quadrat to get total twig length or biomass (from laboratory twig length or biomass measurements), or leaf area (from leaf area measurements), or insect counts (from the field counts) per m^2 of canopy (Form C-17). These values can be extrapolated to numbers per m^2 of the desired landscape area, or wave front, as measured from the remote sensing images (Form C-15).

10. Identification of insect and plant species. Insects and plants whose identification is unknown will be collected, returned to the laboratory, appropriately preserved, and sent to taxonomic specialists for identification and recorded on Form C-18 (COC) for insects and Form C-19 for plants. Identified voucher specimens will be maintained at the Temple ARS laboratory for future reference.

11. Equipment and supplies. Inspection, calibration and location of equipment and supplies will be performed by the ARS technicians as follows: equipment location on Form C-21, instrument calibration on Form C-22, Equipment maintenance and repair on Form C-23, and inspection and storage location of supplies on Form C-24.

12. Landscape vegetation composition and structure. Monitoring of vegetation will be conducted annually during May or June at the release sites for 1 or 2 years before release of the *Diorhabda* beetles and 3 to 5 years after release. These surveys will measure size and occurrence of each vegetation type and species along 1-m wide belt transects extending outward from the release site and later along the creek upstream and downstream as far as the beetles have dispersed or may disperse (Form C-20).

APPENDIX C

List of Forms: Saltcedar Biological Control: Field Release Dispersal Model.

1. List of forms

Form #	Name of form
C-1	Field activities and monitoring log.
C-2	Insect counts in field – per 1m branch
C-3	Foliage density/condition – vigor, visual estimate
C-4	*Diorhabda* dispersal survey – 2-min counts along extended transects
C-5	Probability distribution of *Diorhabda* beetles in quadrats
C-6	Parasites reared from *Diorhabda*
C-7	Predation of *Diorhabda* beetles – caged vs. uncaged in field
C-8	Foliage density/condition – total number 1-meter branches per quadrat
C-9	Foliage density/condition – total branchlets per 1 m branch
C-10	Photograph of each sample branch
C-11	Foliage density/condition – light bar reading
C-12	Beetle behavior – temperature of foliage in top, middle, bottom of quadrats
C-13	Branchlet length, leaf area, condition, biomass
C-14	Weather Monitoring – Downloading data, inspecting instruments
C-15	Remote sensing of saltcedar sites
C-16	Data entry log, year 2006 data
C-17	Branch foliage/condition measure – comparison of methods
C-18	Insect identification log – Chain of Custody
C-19	Plant identification log – Chain of Custody
C-20	Landscape vegetation composition and structure – saltcedar and native vegetation along transects
C-21	Equipment; type, identification, location
C-22	Instrument calibration
C-23	Equipment – General maintenance and repair
C-24	Supplies and expendables – receipt, inspection, where stored
C-25	Field plots – agreements with landowners
C-26	Corrective Action Report (CAR)

2. Examples of Forms Used

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-1. Field activities and monitoring log.

Date _____ 2006 , Location _____

Date	Location	Persons monitoring each day	Type monitoring done	Notes

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-2. Insect counts in field – per 1m branch.

Location: _____ Date: _____ Surveyor name: _____ Weather: _____

				Insect Count – Original Foliage												
				Diorhabda					Other phytophagous*			Predators**				
Transect Quad-	Canopy Strata	Sample Branch #		Egg Mass	Larval instar			Adults	Leaf-hoppers	Scales	Others	Ants	Lady beetles	Assasin Bugs	Others
		This quad	Entire location		1st	2nd	3rd								
	Top	T-1													
		T-2													
		T-3													
	Mid	M-1													
		M-2													
		M-3													
	Bot	B-1													
		B-2													
		B-3													
	Top	T-1													
		T-2													
		T-3													
	Mid	M-1													
		M-2													
		M-3													
	Bot	B-1													
		B-2													
		B-3													

*Phytophage abbreviations: F=white flatid planthopper (*Ormenis* sp.), AL=arctid (hairy) moth larva. Leafhoppers=*Opsius stactogalus*, scales=*Chionapis eutrussa*.

**Predator abbreviations: Con=convergent ladybeetle; C7=7-spotted ladybeetle; TS=twice-stabbed ladybeetle; AG=ash gray ladybeetle; AL=Asian Ladybeetle; Assassin Bugs: ZT=Zelus tetracanthus; ZR=Zelus renardii; SC=Sinea confusa; Others; RBS=red and black stinkbug; BS=mostly black stinkbug; GS=gray Stinkbug (Brochymena); S=Spider; GL=green lacewing; BL=brown lacewing.

Form C-3. Foliage density/condition – vigor, visual estimate. Location ____________________, Date _______________,
Surveyor names _____________________, Weather _____________________

				Foliage vigor Original growth				% regrowth of total		Foliage vigor regrowth				
Transect, Quad	Distance from release point	Canopy section	Metal branch #	% Green	% Yellow	% Brown	% Dead	Each branch	Entire Quad.	% Green	% Yellow	% Brown	% Dead	Visual estimate defoliation %
		Top	T-1											
			T-2											
			T-3											
			Entire top											
		Mid	M-2											
			M-2											
			M-3											
			Entire middle											
		Bot	B-1											
			B-2											
			B-3											
			Entire Bottom											
		Top	T-1											
			T-2											
			T-3											
			Entire top											
		Mid	M-2											
			M-2											
			M-3											
			Entire middle											
		Bot	B-1											
			B-2											
			B-3											
			Entire Bottom											
		Top	T-1											
			T-2											
			T-3											
			Entire top											
		Mid	M-2											
			M-2											
			M-3											
			Entire middle											
		Bot	B-1											
			B-2											
			B-3											
			Entire Bottom											

Form C-4. *Diorhabda* dispersal survey – 2-min counts along extended transects.

Location _____, Date _____2006, Name of sampler _____, Weather conditions _____

			Number *Diorhabda* counted in 2 min.				
Transect #	Tree #	Distance from origin	Eggs	Larvae	Adults	Tree condition	Notes

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-5. Probability distribution of *Diorhabda* beetles in quadrats.

Location _____ **Date** _____**2006** **Surveyor** _____ **Weather** _____

Transect, Quadrat # & population level % % defoliation	Canopy section	Insect Stage	Branch # 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	TOP	Eggs																				
		Larvae 1																				
		Larvae 2																				
		Larvae 3																				
		Adults																				
	MED	Eggs																				
		Larvae 1																				
		Larvae 2																				
		Larvae 3																				
		Adults																				
	LOW	Eggs																				
		Larvae 1																				
		Larvae 2																				
		Larvae 3																				
		Adults																				
	TOP	Eggs																				
		Larvae 1																				
		Larvae 2																				
		Larvae 3																				
		Adults																				
	MED	Eggs																				
		Larvae 1																				
		Larvae 2																				
		Larvae 3																				
		Adults																				
	LOW	Eggs																				
		Larvae 1																				
		Larvae 2																				
		Larvae 3																				
		Adults																				

OBSERVATIONS:
*High, medium or low beetle population density on sample trees.

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-6. Parasites reared from *Diorhabda*.

Site location _____, Date collected _____2006, Person collecting _____

	Saltcedar				Number reared in lab			ID by specialist	
Beetle stage collected	level defoliation	Trans, Quad	Strata of tree	No. coll.	Beetle next stage	Date emerged	Parasite sp. ID	Spec #1 ID	Notes*

* Date transferred to Form C-18 (Insect Identification log)

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-7. Predation of *Diorhabda* beetles – caged vs. uncaged in field.

Location ______________ Date__________2006 Beetle stage_______________

	Beetles placed in test			Examined			
Cage #	Date	Number in cage	Number out of cage	Date	Surviving in cage	Surviving out of cage	Notes

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-8. Foliage density/condition – total number 1-meter branches per quadrat.

Transect # Quadrat #	Canopy strata	Total number 1-m branches	Notes
	Top		
	Middle		
	Bottom		
	Top		
	Middle		
	Bottom		
	Top		
	Middle		
	Bottom		
	Top		
	Middle		
	Bottom		
	Top		
	Middle		
	Bottom		
	Top		
	Middle		
	Bottom		
	Top		
	Middle		
	Bottom		
	Top		
	Middle		
	Bottom		
	Top		
	Middle		
	Bottom		

Saltcedar Biological Control: Field Release: Dispersal Model - Form C-9. Foliage density/condition – total branchlets per 1 m branch.

Location: ____________________, Date: ____________________, Recording persons: ____________________

Transect # Quadrat #	Canopy Strata	Position in quad	Sample branch #	Stem lengths > 25 cm/per 1 m branch	Total cm/1-m branch
	Top	F			
		L			
		R			
		B			
	Middle	F			
		L			
		R			
		B			
	Bottom	F			
		L			
		R			
		B			
	Top	F			
		L			
		R			
		B			
	Middle	F			
		L			
		R			
		B			
	Bottom	F			
		L			
		R			
		B			
	Top	F			
		L			
		R			
		B			
	Middle	F			
		L			
		R			
		B			
	Bottom	F			
		L			
		R			
		B			

*Measure length of all leaf bearing stems (generally at least 1 mm diameter).
**Front edge is towards origin of transect; Left and Right edges are oriented from standing with back toward transect origin and facing front edge; Back edge is away from origin of transect.

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-10. Photographs of each sample branch.

Location ______________________, Date____________2006,

Persons photographing ______________________

	Branch #			
Transect, Quad	This tree	Entire location	Neg #	Notes

Saltcedar Biological Control: Field Release: Dispersal Model
Form C-11. Foliage density/condition – light bar reading.

Date: _____2006, Weather: _____,

Location: _____, Surveyor name: _____

Trans. % Quad.	Trees within quadrat	Time of day	Number readings & average	Under canopy	Open Sky	% Int.

*Average of 40 readings in each quadrat, all in shade. Do in selected quads only.

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-12. Beetle behavior – temperature of foliage in top, middle, bottom of quadrats.

Date _____2006, Person recording _____, Weather _____

Transect, Quad #	Canopy Strata	Time of day		Temperature (10 readings per quadrat)				Notes
				Individual readings		Mean		
		Hr	Min	Exposed	Shaded	Exposed	Shaded	
	T							
	M							
	B							
	T							
	M							
	B							
	T							
	M							
	B							
	T							
	M							
	B							
	T							
	M							
	B							

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-13. Branchlet length, leaf area, condition, biomass.
Location _____, Date collected _____2006,
Persons collected _____ , Persons _____ in lab _____

Field data										Laboratory data													
Transect# Quadrat #	Distance/ direction from quadrat	Level in tree (T, M, B) and branch #	Photo # of branch	Visual est. of % damage			Total length branchlets > 25 cm			Total length branchlets > 25 cm			Total length all branchlets			By leaf area meter (cm^2)				Biomass(g)			
				g	y	b	g	y	b	g	y	b											

[a]Measure branch in field from tree near sampling quadrat, measure by standard field procedure, remove branch and return to lab; measure length of all tiny branchlets, separated by green, yellow, dead; measure area of all branchlets (g,y,d & stems) with leaf area meter, dry all in oven and get biomass of g, y, by stems.

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-14. Weather Monitoring – Downloading data, inspecting instruments.

Site Location _____ , **Date** _____**2006** , **Person inspecting** _____

Instrument Location							
Main station	Transect, Quadrat	Date	Condition	Date	Condition	Date	Condition
Hobo 1							
H-2							
H-3							
H-4							
H-5							
H-6							
H-7							
H-8							
H-9							
H-10							

Saltcedar Biological Control: Field Release: Dispersal Model

Form C-15. Remote sensing of saltcedar sites.

Type image _____ **Agency doing survey** _____

				Canopy area of saltcedar present (m^2)		
Date photographed	Location	Area covered in image	Altitude of photo (ft)	Defoliated by *Diorhabda*	Not defoliated	Images stored at:

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Form C-16. Data entry log, year 2006 data.

Location of field plot _____, or type experiment _____

Type data	Ref. ID (dates of data, etc.)	Data entered		Verified		Date backed-up	Where stored
		Date	Person entering	Date	Person		

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Form C-17. Branch foliage/condition measure – comparison of methods[a]

Location _____ **Date collected** _____

		Visual estimates % of total foliage		Total 1-m branches per quad (T, M, B)	Branchlet lenghth > 20 cm (cm)		Photo each branch (3 level/quad)	Leaf area meter – cm^2 each branch (date)	Total length all branchlets	Biomass each branch		Light bar
T, Q	Branch#[a/]	Original g, y, b	Regrowth g, y, b		Original	Regrowth	% green/yellow/dead	green/yellow/dead/stems		Dry wt (g)		(0/Quad)
										Foliage		
	T-1											
	T-2											
	T-3											
	M-1											
	M-2											
	M-3											
	B-1											
	B-2											
	B-3											
	T-1											
	T-2											
	T-3											
	M-1											
	M-2											
	M-3											
	B-1											
	B-2											
	B-3											

[a/] Branch selected out of quadrat but nearby, used for destructive sampling, 3 branches each level in tree. T=top 1/3 of tree, M=middle 1/3, B=bottom 1/3 of tree.

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Form C-18. Insect identification log – Chain of Custody.

Sent to specialist for ID: Specialist Name _____, Address _____,
Date Sent _____ 2006

	Collected			Prelim ID		Identification by specialist					
Specimen no.	Where	Date	Description	Taxonomy	By:	Date returned	No. kept	No. returned	ID Genus / Sp.		Location of voucher specimens

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Form C-19. Plant identification log – Chain of Custody.

Sent to specialist for ID: Specialist Name _____ , Address _____ ,

Date Sent _____ 2006

	Collected:				Prelim ID		Identification by specialist				
Specimen #	Location	Date	GIS	Tree tag #	Taxonomy	By:	Date returned	No. kept	No. returned	ID Genus sp.	Location of voucher specimens

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Form C-20. Landscape vegetation composition and structure – saltcedar and native vegetation along transects.

Site location _____ , Persons doing monitoring _____

Transect location, length, direction	Distance along transect	Shrub/tree species Canopy, diam, ht			Forbs and grasses	
		Species	Canopy diam (m)	Ht (m)	% Canopy cover	Height (cm)

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Form C-21. Equipment; type, identification, location.

Equipment name	Mfg, model, SN	Location	Backup and spare parts

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Form C-22. Instrument calibration.

Instrument type	Date calibrated	Calibrated by whom

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Form C-23. Equipment – General maintenance and repair.

Equipment type	Date of maintenance	Person inspecting	Condition	Action taken, by whom

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Form C-24. Supplies and expendables – receipt, inspection, where stored.

		Condition		
Date received	Inspected by	Count	Condition	Corrective action if needed

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Form C-25. Field plots – agreements with landowners.

Location of plot	Landowner name and address	Date of agreement	Location of file copy

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Form C-26. Corrective Action Report (CAR)

CAR #: _____

Report Initiation Date: _____ **Area/Site:** _____

Reported by: _____ **Analyte/Activity:** _____

State the nature of the problem, nonconformance or out-of-control situation:

_____

_____

_____

Affected sample #s / date(s) of sample collection: _____

Project(s): _____ Attached documentation: NA COC FDS SampLink Flow8

Possible Causes and Corrective Actions Taken / Recommended:

_____

_____

_____

CAR routed to: _____ Date: _____

Supervisor: _____

Circle one: **Tier 1** (does not affect final data integrity) **Tier 2** (possibly affects final data integrity)

Corrective Actions (If actions are to be taken, include Responsible Party[1] and proposed completion date, where appropriate)

For specific incident: Action to be taken _____

_____

_____

To prevent recurrences: Action to be taken _____.

_____

_____

Effect on data quality: _____

Responsible Supervisor: _____ Date: _____

Concurrence: _____

Program/Project Leader: _____ Date: _____

Program/Project Quality Assurance Officer: _____ Date: _____

TSSWCB Quality Assurance Officer: _____ Date: _____

[1] Party responsible for implementing corrective action is also responsible for notifying QAO of completion and outcome of corrective action.