Texas State Soil and Water Conservation Board
FY03 CWA Section 319(h)
TSSWCB Project #03-11

NONPOINT SOURCE SUMMARY PAGE

1. Title of Project: Upper Colorado Saltcedar Control Project: Biological Control Component

2. Project Goals/Objectives: The goal of this project is to aid in implementing the Implementation Plan for Sulfate and Total Dissolved Solids TMDLs in the E.V. Spence Reservoir by biologically treating saltcedar in the Colorado River Basin in an effort to reduce nonpoint source (NPS) pollution loadings resulting from invasive brush species on agricultural lands.

3. Project Tasks: (1) To introduce *Diorhabda elongata*, Chinese leaf beetle, to the E.V. Spence Reservoir as a means to control saltcedar; (2) To promote project participation and public interest in the project; (3) To monitor the success of the leaf beetle using satellite imagery.

4. Measures of Success: (1) To demonstrate the effectiveness of the Leaf Beetle to control saltcedar in the E.V. Spence Reservoir Basin; (2) To provide sustainable saltcedar control in the E.V Spence watershed and Colorado River.

5. Project Type: Statewide ( ); Watershed (X); Demonstration ( ).

6. Waterbody Type: River (X); Groundwater ( ); Other ( ).

7. Project Location: E.V. Spence Reservoir (Segment 1411); Colorado River above Lake J.B. Thomas (Segment 1412).

8. NPS Management Program Reference: *Texas Nonpoint Source Pollution Assessment Report and Management Program* approved October 1999.

9. NPS Assessment Report Status: Impaired (X); Impacted ( ); Threatened ( )

10. Key Project Activities: Hire Staff ( ); Monitoring (X); Regulatory Assistance ( ); Technical Assistance ( ); Education (X); Implementation (X); Demonstration (X); Other ( )

11. NPS Management Program Elements: Milestones from the *1999 Texas Nonpoint Source Pollution Assessment Report and Management Program*, which will be implemented include: (1) coordinating with federal, state, and local programs; (2) committing to technology transfer, technical support, administrative support, and cooperation between agencies and programs for the prevention of NPS pollution.

12. Project Costs: Federal ($99,246); Non-Federal Match ($0); Total Project ($99,246)

13. Project Management: Texas State Soil and Water Conservation Board (State Board). Cooperating Entities: USDA-ARS

14. Project Period: Three years from start date.

I. TITLE: UPPER COLORADO RIVER SALTCEDAR CONTROL PROJECT: BIOLOGICAL CONTROL COMPONENT

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30 January 2003

II. 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 plant nursery 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 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.; 4 of them, and their hybrids (Gaskin and Schaal 2002), cause almost all of the damage (reviewed by DeLoach et al. 2000, 2003).

A. 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, increase soil salinity and 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 fish, 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, insects and other invertebrate and plants have been severely affected (reviewed by DeLoach and Tracy 1997, DeLoach et al. 2000).

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), and especially between it and saltcedar by DeLoach and Tracy (1997), DeLoach et al. (2000), and Dudley et al. (2000). 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, cowbird predation, need for free water in streams, lakes or flooded areas, lack of proper food (insect larvae), lethal high temperatures, and possibly stress on the females. 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 south of Topock Marsh is probably caused by the saltcedar invasion and that temperatures for the willow 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 et al. 2000; manuscript submitted 2002).

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 Merideth 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).

B. Depletion of Water Resources for Agriculture and Municipalities

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. 1970, 1982), the middle Rio Grande (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 (in Brown et al. 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).

Below average rainfall over the past decade, together with saltcedar depletion of stream flow (estimated at one-third of the total allowable annual depletion of riverflow, has created urgent water shortages for agriculture and municipalities throughout the southwest. This has resulted in default of water agreements between states and between the United States and Mexico, with serious economic and political consequences. Large-scale and expensive saltcedar Aeradication 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.

Along the Rio Grande, one-third of the allowable annual depletion of water is lost to saltcedar (Steve Hansen, 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 Carter, personal communication). The present severe drought has reduced the streamflow available for irrigated agriculture and municipal use, threatening the livelihood of farmers, causing water rationing in towns and cities. Flow from the Rio Grande no longer reaches the Gulf of Mexico.

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 take 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).

C. Causes of the Saltcedar Invasion

The invasion of saltcedar is thought by many to be caused mostly by abiotic or human produced environmental changes dam building, livestock grazing, groundwater pumping, etc. and that the invasion was only passive and followed these changes (Everitt 1998, reviewed by DeLoach et al. 2000). Its 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 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 (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 saltcears allow it to interest 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 that constantly suppress native plant communities but they do not damage saltcedars (DeLoach et al. 2000).

D. 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 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 1989, 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 Rivers 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 helicopters. 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 (Hart et al. 2000).

E. Appropriateness of Biological Control for Saltcedar

1. 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, the opposite of the objective for herbicides 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. In ”Augmentation”, methods are developed for increasing the numbers of control agents, such as by mass rearing and release. 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, and as reviewed by DeLoach 1997). 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). 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).

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 130 weed species in 51 countries, and using 272 introduced control agents since 1865. 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. 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. (Julien and Griffiths 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, Old World climbing fern, Brazilian pepper tree, yellow starthistle, houndstongue, toadflax, some knapweeds, and, hopefully, saltcedar.

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 and Harris 1982), 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). Test 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 (Zwölfer and Harris 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.

2. 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 1989, 1991, 1996; DeLoach and Tracy 1997).

Biological control offers the potential for effective control of saltcedar. It is highly specific to saltcedar and can control only it in mixed stands without damage to any other plants. It also 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) 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 (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.

Any possible 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 disadvantage of biological control is 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 (if the beetles are as effective as indicated in recent field tests) if they are redistributed manually. 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. Two years after the release of *D. elongata* into the open field at the 6 most northern sites in Colorado, Wyoming, Utah, Nevada and California, where it is adapted to the long summer daylength, *D. elongata* attained high populations at 5 sites and it has produced severe defoliation of saltcedar at 2 sites. These results (and those of several other successful biological control of weeds projects) indicate that biological control is potentially capable of controlling saltcedar in all situations from monotypic stands of large trees to dispersed or mixed stands of large or small trees. In several other successful projects, biological control was the only control used and herbicidal or mechanical controls were unnecessary.

In situations of acute water shortage such as exist in Texas and the other Southwestern states where rapid control is essential, biological control is the method of choice a) to follow herbicidal treatments to control regrowth and reintroductions of saltcedar, b) to use 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, c) for use in areas where herbicides are unlikely to be used over the next 3 or 4 years, and d) 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.

III. PREVIOUS RESEARCH ON BIOLOGICAL CONTROL OF 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 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 attach 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 1989, 1990; 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).

The *Diorhabda elongata* beetles have good potential for highly effective, safe and cost-efficient control of saltcear. 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., 2003a; Lewis et al. 2003a).

These tests, and a summary by DeLoach et al. (2003b) 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 this plant in the field. 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, was only 10 to 20% 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 1).

Table 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 (no. replications)		
Test plant	Larval survival[a] egg to adult	Adults on plants[b]	Eggs laid on plants[b]
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]About larval tests.
[b]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). From Lewis et al., 2003 (Biological Control, May-June 2003).

Diorhabda elongata deserticola 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 from the summer of 1999 and 2000 at 10 sites in Texas, Colorado, Wyoming, Utah, Nevada and California. It successfully overwintered and heavily damaged saltcedar 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) and at Seymour. Beetle populations developed in the surrounding saltcedar plants at Pueblo, CO, Lovell, WY, Delta, UT and Lovelock, NV.

At Lovelock, NV these 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. The anticipated even larger 2nd generation in August is expected to defoliate an ever larger area. During the spring of 2003, most saltcedar plants had resprouted from the base, and some had resprouted from the upper branches but most of the upper stems had died. During June, adults and larvae had killed mostof this regrowth. In the previous field cages, defoliation for 2 years completely killed even larger plants. At Lovelock, plant kill may exceed 95% within 3 years after release of the beetles. However, at other locations (especially at Lovell, WY) predation by ants has seriously reduced the effectiveness of the beetles. 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.

Populations at Pueblo, CO produced extensive defoliation of ca. 35 nearby plants in 2002 and have extended defoliation to a larger area in 2003.

B. Failure of Fukang/Chilik beetles in Texas.

The *D. e. deserticola* beetles did not overwinter in cages at Seymour, Dallas or Temple, TX nor after release into the open field at Seymour, Schurz or Bishop. 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 7 months before saltcedar foliage becomes available in March. These observations were confirmed by our collaborator 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 diapause; maximum daylength at Seymour (33.3ºN) at the summer solstice is only 14 hr. 21 min., is somewhat less at Dallas, and is 14 hr 10 min at Temple (31.1ºN). We conclude that these beetles will not control saltcedar in Texas or in other locations south of ca. 38ºN latitude (Lewis et al. 2003 in press; Dan Bean, USDA-ARS, Albany, CA, personal commu.).

C. Potential of other *Diorhabda* biotypes 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; Tunis, Tunisia and Karshi, Uzbekistan. In laboratory tests at Albany, all 4 of the new biotypes appear to be adapted to short daylength south of the 37th parallel. During the fall of 2003, we plan to release the Crete, Turpan and Karshi beetles into field cages at Lake Thomas and/or Beal's Creek to determine which overwinters, develops best, and damages saltcedar the most there. Then, the best biotype will be released into the open field during the spring of 2004.

The Crete beetles were collected along the north shore of Crete, at 35º28'N latitude, or similar to that of Amarillo, TX. These appear slightly different morphologically from *D. e. deserticola* and may be a different subspecies. 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*. The second shipment was of *D. e. deserticola* (the same subspecies as the Fukang beetles) but collected near Turpan, China only 100 miles southwest of Fukang. These beetles appear to be identical to the Fukang beetles in every way except for daylength response.

More recent tests, conducted during June and July 2003, compared *D. elongata* beetles from Crete, Turpan, Uzbekistan and Tunisia individually but at the same time in paired plant tests (1 saltcedar and 1 *Frankenia* plant together) in small cages outdoors at Temple. These beetles were strongly attracted to saltcedar (Table 2), laid several eggs on the cage walls, but placed very few or no eggs on *Frankenia* (Table 3).

Table 2. Host selection by adult *Diorhabda elongata*: Paired-choice adult tests, Temple, TX, July 2003 (preliminary results)[a]

	Mean no. adults per plant for each beetle type			
Location of adult beetles	Crete, Greece	Tunis, Tunisia	Karshi, Uzbekistan	Turpan, China
Test 1	*T. ramosissima* vs. *F. jamesii*			
Tamarix ramosissima (CO)	**16**	**13.5**	**15**	**10.5**
Frankenia jamesii (CO)	**0**	**0**	**2**	**0.5**
Cage walls	0	3.5	3	1
Missing/dead	4	3	0	8
Test 2	*T. ramosissima* vs. *F. jamesii*			
Tamarix ramosissima (CO)	**16**	**13.5**	**15**	**5.5**
Frankenia johnstonii (TX)	**1**	**0**	**2**	**0**
Cage walls	0	5	2	3
Missing/dead	3	1.5	1	11.5

[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*), 1 or 2 replications of each test/beetle type completed to date.

Table 3. Oviposition host selection by female *Diorhabda elongata*: Paired-choice adult tests, Temple, TX, July 2003 (preliminary results)a

	Mean no. eggs per plant for each beetle type			
Location of eggs	Crete, Greece	Tunis, Tunisia	Karshi, Uzbekistan	Turpan, China
Test 1	*T. ramosissima* vs. *F. jamesii*			
Tamarix ramosissima (CO)	**459**	**621**	**603**	**609**
Frankenia jamesii (CO)	**0**	**0**	**0**	**0**
Cage walls	36	14	42	8
Test 2	*T. ramosissima* vs. *F. johnstonii*			
Tamarix ramosissima (CO)	**235**	**502**	**242**	**289**
Frankenia johnstonii (TX)	**0**	**0**	**23**	**0**
Cage walls	35	18	13	73

[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*), 1 or 2 replications of each test/beetle type completed to date.

Both the Crete and the Turpan beetles remained active throughout the growing season at Temple and Dallas. At Temple, they oviposited until mid September and were still active in the cages until mid-November. These beetles appear to be insensitive to photoperiod and probably begin diapause in response to cold temperatures in the fall. The Crete beetles overwintered in outdoor cages under nearly natural conditions at Temple, TX with little mortality, emerged during March, and began vigorous egg laying after about a week. The first spring generation of larvae began pupating in early May, about a month earlier than in the northern sites. Only a few Turpan beetles were available and they have not yet overwintered. The Turpan beetles increased to high populations in the field cages at Seymour, TX during July 2003 and now are ready for field release at Lake Thomas.A Letter of Concurrence from FWS was obtained on ____ June and Release Permits from APHIS were obtained on 2 July for release at all requested sites in Texas including Seymour, Merideth 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. Previous projections (DeLoach and Tracy 1997) suggested that 75 to 85% control of saltcedar was sufficient to salvage most of the water losses and to allow essentially full recovery of native plant and animal communities.

Effectiveness. The Fukang/Chilik beetles released into the open field in Nevada and Colorado increased rapidly and during August populations of severals tens of thousands of second generation beetles completely defoliated a dense stand of saltcedar in an area of about 2 acres. The defoliated areas quadrupled in size. The first generation beetles in July 2003 nearly quadrupled the defoliated area and a further large increase in control is expected from the second generation larvae in August 2003. Similar control has occurred near Pueblo, CO but in a less dense stand. However, control in other locations has been somewhat restrained by ant and bird predation. Early results indicate that this could be one of the most effective biological control programs ever initiated. Monitoring is essential to determine the effect of this degree of control in improving the native plant and animal communities and to determine if the beetle populations are being restricted by predators or other factors.

The behavior in the open field of the Turpan and Crete beetles planned for release at Lake Thomas/Beal's Creek is still unknown, and is the objective of the present 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, and is a good indication that they can reproduce throughout the summer (the Fukang adults did not lay eggs after June) and can overwinter (the Fukang beetles did not overwinter). These experiments indicate that both the Crete and the Turpan beetles can establish and can control saltcedar in the climatic/daylength zones of Texas, unless suppressed by naturally occurring biotic agents.

Monitoring. The major cost of this Proposal is for monitoring the increase, dispersal, mortality factors, seasonal abundance and effectiveness of the beetles in controlling saltcedar; and the effect of control in recovery of native vegetation and wildlife populations. This monitoring is required by our Letter of Concurrence from FWS and our release permits from APHIS. The few (300 to 500) beetles to be released are expected to increase to hundreds of thousands, to disperse throughout the floodplain of Lake Thomas, and to control the saltcedar over the original sample area within the 3 years of this study, and to be poised to control saltcedar in the remainder of the Texas Colorado River watershed. This can occur at extremely little cost of only a few thousand dollars needed to redistribute the beetles from sites of abundance, from which the beetles spread naturally into nearby areas. The release site for the Fukang beeltes near Lovelock, NV now seems poised for just such a degree of control throughout the saltcedar infested Humboldt River and Basin, and in only 3 years since their initial release in May 2001. On the other hand, certain biotic factors such as predation (especially by ants) and parasitism, or lack of adaptability to the climate/ecology of this region, may seriously reduce their effectiveness. The monitoring program is essential to determine which direction control will go and to determine the cause and how to correct it if control is not successful.

Monitoring of the beetles and their reproduction, mortality and effect on saltcedar, of vegetation structure and composition, and of bird and butterfly species diversity and populations in both native and monotypic saltcedar, and mixed native/saltcedar communities, was conducted at Seymour during the 2002 growing season, and methodologies are now well developed. Remote sensing of the Seymour site has been done now for 2 years.

Quality Assurance Project Plan (QAPP). A QAPP will be submitted to EPA through TSSWCB at least 60 days prior to beginning of any sampling being conducted under this grant. The plan will be based on the "Plan for Monitoring the Effects of Releasing the Saltcedar Leaf beetle, *Diorhabda elongata*, for Biological Control of Saltcedar: *D. elongata*, Vegetation, and Wildlife, Research Phase, Stage B", prepared by the Insect, Vegetation and Wildlife Subcommittees of the Saltcedar Consortium, 23 October 2000, and the Revised Vegetation Monitoring Protocol of April 203 (attached).

IV. PROPOSED ACTIONS: Release of the leaf beetle, *Diorhabda elongata deserticola* for Biological Control of Saltcedar

A. Location to be Released

The beetles will be released within "Segment 1" of the Colorado River Saltcedar Project, which includes saltcedar infested areas of the Colorado River, TX and its tributaries from Lake Thomas dam to their headwaters, as follows:

1. One site within the Lake Thomas shoreline or delta or nearby upstream (Fig. 3).
2. A subsite along Beal's Creek, 25 miles south of Lake Thomas and just east of Big Spring (Fig. 4).
3. After the first year, unlimited releases along the Colorado and its tributaries from Lake Thomas dam to their headwaters.
4. During Year 5, beetles will be released throughout "Segment 3" to obtain control of regrowth and reinfestation.
5. The circumscribed area into which the beetles are allowed to disperse without requirement to eradicate them, includes the Colorado River of Texas and its tributaries from Lake Thomas Dam to their headwaters, and from Lake Thomas Dam downstream to Lake Buchannan Dam (or as far as saltcedar extends).

The Lake Thomas sites is part of a larger group of one existing site at Seymour, TX and 7 Texas sites and 3 New Mexico sites being requested of Region 2, U.S. Fish and Wildlife Service, Albuquerque, NM, and 8 other sites within Regions 1 and 6. The location (and justification) for the Texas sites is as follows:

1) Big Spring/Lake Thomas (Upper Colorado River control program, climatic adaptations of the beetle, far from sw WIFL).
2) Lake Meredith (Bureau of Reclamation and National Park Service revegetation program,

climatic adaptation).
3) Candelaria (Rio Grande control program, climatic adaptation).
4) Big Bend National Park (Rio Grande control program, and NPS/Bureau of Reclamation revegetation program, climatic adaptation).
5) Zapata (Rio Grande control program, climatic adaptaion).
6) Kingsville, TX (climatic adaptation).
7) San Jacinto State Park (climatic adaptation in a humid area, control in areas far from sw WIFL).
8) Seymour, TX (already an approved site, Wichita River).

B. Purpose of Releases

The purposes of the site along the upper Colorado River of Texas are 1) to demonstrate the advantages and desirability of integrating biological control with herbicidal control in the Colorado River Saltcedar Project, 2) to demonstrate the capacity of the beetles to effect control without herbicides, 3) to demonstrate the climatic adaptation and effectiveness of the short-daylength adapted *Diorhabda elongata* beetles obtained from Tupran, China; Crete, Greece; and Karshi, Uzbekistan; and 4) to implement control of saltcedar in areas far removed from possible influences on the southwestern willow flycatcher.

C. Species/biotype to be Released

Along the Colorado River of Texas and its tributaries upstream from Lake Thomas dam (initially along Lake Thomas and Beal's Creek) (near Big Spring) we propose to release the biotypes of *Diorhabda elongata* from Crete, Turpan and Karshi into field cages into field cages during the fall of 2003. The biotype that overwinters, reproduces, and that controls saltcedar best will then be released into the open environment during the spring of 2004.

Laboratory tests at Albany, CA demonstrated that all these biotypes require substantially less than the ca. 14 to 14 hr 45 min daylength of this area and so should be adapted to overwinter successfully here. In the outdoor cages at Temple, the Crete beetles laid eggs through September and remained active through October, overwintered with little mortality during the 2002/2003 winter, and reproduced well the following spring. Overwintering ability of the other 2 biotypes has not yet been demonstrated in outdoor cages. Our tests demonstrate that all these biotypes are safe to release, having the same behavior and host range (Tables 2 and 3) as the Fukang/Chilik beetles previously released (Table 1).

D. Source of Insects to be Released

Beetles for release may be obtained from 2 sources. First, adults, eggs or larvae from overwintering cages at Seymour, Dallas and Temple, Texas will be released, if sufficient numbers are available. If not enough, these beetles may be allowed to multiply and the following generation may be released. These beetles were obtained from shipments received from overseas, reared in quarantine, and are free of pathogens, parasites or other arthropod species prior to release. Second, beetles may be obtained from laboratory cultures at Albany, CA that also are free of pathogens, parasites or other organisms.

E. Numbers of Insects to be Released

At each site, we will release 100 to 200 overwintered adult *D. elongata* in each of 2 cages, with repeated similar releases if needed, to obtain at least 50 to 100 reproducing females in each cage. As soon as authorizations are obtained from FWS and APHIS, releases into the open field will be made at one point at each of Lake Thomas and Beal's Creek during the first year, with 500 to 2000 adults and/or large larvae released at each site. During the second and third year, additional secondary releases of 200 to 500 beetles will be made at sites throughout the area upstream from Lake Thomas dam (after final clearance by FWS and APHIS) limited only by the availability of beetles and personnel to distribute them.

F. Cages and Surroundings

The beetles first will be released into field cages at each site. Cages are made of 32-mesh Saran plastic screening, placed over a metal frame, and entered through a zippered door on one side. The cages are 10x10 by 6 to 10 ft high. The bottom of the screen is sandwiched between two 1x6 boards bolted together, buried 4 inches in the soil, backfilled with the soil and tapped firmly in place; this prevents escape from under the cage. The cages are surrounded by a minimum of a 4-strand barbed-wire fence 4 ft high at least 4 ft from the cage on all sides to prevent cattle or wildlife from reaching the cages. If other animals are present (such as feral hogs), then ”hog panels” or chain-link fence will be used. Some of the initial releases will be inside nylon mesh sleeve bags tied over branch terminals inside the big cages.

The field cages will be located in a stand of saltcedar, or mixed saltcedar/native vegetation, of sufficient extent that beetle dispersal, effect on saltcedar stands, and recovery of native vegetation after control can be monitored. The cages will be located in an area that does not flood, that has limited access by the public, and that is hidden from view by the public. The owner or manager will agree not to apply herbicides or insecticides, or to use mechanical controls or fire to control weeds or brush on the site, or in the nearby area that might adversely affect the beetles in the cages or after release from the cages. The area of saltcedar should extend at least to 1 to 3 miles upstream and downstream, along a lakeshore, or in radius, and should be in an area where saltcedar is dense and extensive enough to be damaging to the native vegetation but where sufficient native seed trees are present to allow rapid revegetation.

G. Schedule and method of releases

1. General. In year 1 we will determine the exact location of the release cages, obtain landowner/manager agreements, and cut back the saltcedar shrubs to 2-3 ft high, to promote new shoot growth in the spring. Releases will be made as soon during the spring of the first year as overwintering adults emerge from laboratory cultures or from outdoor nursery sites, which normally occurs from late April to mid-May in nature, and as soon as release permits are obtained. A small to moderate-sized reproducing population will be maintained inside the cages at each new site throughout the 1st year growing season and through the following winter to determine overwintering and as a back-up population in case the released beetles don’t immediately establish. These beetles will be transferred to other adjacent cages during the growing season if needed to maintain sufficient good-quality foliage as food for the beetles. Releases can be made as late in the year as the beetles are still active (September or October, based on observations in outdoor cages at Temple) and still allow for the beetles to successfully overwinter.

After the released and overwintered adults have produced larvae and pupae, the 1st generation adults (usually in late June) inside the cages will be released outside the cages and onto healthy plants near the cages. If larvae produced by the overwintering adults are numerous, part of the large 3rd instars also may be released outside the cages. Some of the initial releases into the cages, and of the initial releases outside the cages, will be into sleeve bags placed over the terminals of branches so that oviposition, duration of stages, mortality and rate of increase may be measured. These bags will remain in place until adult oviposition can be confirmed, or if medium-sized 3rd instars are produced; 3rd instars should be allowed to pupate naturally on the soil surface, or in special pupation cages on the soil surface.

After establishment and population increases in the field cages, secondary releases will be made throughout the saltcedar infested areas upstream from Lake Thomas Dam. These releases will be of 100 to 500 adults or large larvae placed on open trees in a 10 m diameter area, and not necessarily in cages. The objective is to demonstrate for the first time the effectiveness of biological control of saltcedar on a small riverbasin sized area.

2. Year by Year Activities. The detailed schedule of year by year activities of releases, monitoring and integration into overall control program are as follows:

Year 1
Segment 1: Release 50 to 200 beetles into each of two 10x10x6 ft field cages; monitor populations of eggs, 1st, 2nd, and 3rd-instar larvae, and adults weekly. Record predators (ants, spiders, predaceous bugs, and others) and destroy as many as possible. Record other insects feeding on the saltcedar plants or on native shrubs within the cage. If insect populations increase to the point of threatening their food supply, transfer 25 to 50 adults to a new nearby cage. When adults of the 1st generation emerge in mid to late June, place 10 males and 10 females in each of 6 sleeve bags over branches outside the cage; record numbers of eggs laid each week, move beetles to a sleeve cage on a fresh branch and count the eggs again each week until the beetles die. Repeat this process during each generation during the growing season. This is to determine the seasonal cycle and number of generations of the beetles throughout the growing season and the date they enter overwintering diapause (if any).
In April, establish point counts for monitoring birds in riparian habitats, 10 points within saltcedar in or near the treatment area and 10 points in a nearby untreated control area of native vegetation.

The vegetation monitoring layout and pre-release vegetation monitoring was done on 28-29 July 2003 at Lake Thomas; 40 marked saltcedar trees were established within a 10 ha sampling area, as specified in the Vegetation Monitoring Plan (Figs. 1 and 2). Monitor plant size, plant condition, and foliage quantity on 4 each 40 cm long branch terminals, percent canopy cover of each grass and forb species in two 1-m square quadrats (one at the trunk and one at the canopy dripline) under each of 40 trees and distance to, size and species of the 5 nearest neighbors of the 40 trees.

Segment 3: Obtain baseline data on vegetation and wildlife monitoring as in Segment 1.

Year 2
Segment 1: In March and April, record data and numbers of overwintering adult beetles emerging within the cages each week. This is to determine the date and size of the overwintering adult population and the health and fecundity of the overwintering females. Measure dispersal of beetles from original release point and damage caused to saltcedar. Repeat vegetation and wildlife monitoring as in Year 1.

Segment 3: No monitoring.

Year 3
Segment 1: Repeat monitoring as in Year 2.
Segment 3: Repeat vegetation and wildlife monitoring as in Year 1.

V. CLEARANCES AND AUTHORIZATIONS REQUIRED

A. Procedures

In order to release exotic biological control agents in the United States to control weeds, several authorizations are required under Federal laws and regulations. Clearances for these actions must be obtained through the Department of Agriculture of each state where releases are desired, and then from the U.S. Department of Agriculture's Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ), through petition to the APHIS-PPQ's Technical Advisory Group for Biological Control Agents of Weeds (TAG). The TAG consists of 13 members from USDA, Department of Interior and other Federal Agencies, and also sends petitions for review to their Canadian and Mexican counterparts. If any endangered species may be affected by the releases, the TAG member from the USDI Fish and Wildlife Service advises APHIS and the petitioner that a Biological Assessment to FWS will be required for FWS review and approval via a Letter of Concurrence (or a Biological Opinion) as authorized under Section 7 of the federal Endangered Species Act (ESA). If approved, APHIS-PPQ then will publish an Environmental Assessment (EA) in the Federal Register for public comment. After review of the public comments, APHIS will publish a Finding of No Significant Impact (FONSI) if their review indicates that the release is justified. After these approvals, APHIS-PPQ issues release permits through each state Department of Agriculture, to the petitioner.

B. Previous Clearances Obtained

All regulatory clearances have been obtained for release of *Diorhabda elongata* into the field in the United States, including Texas, as required under USDA-APHIS-Plant Protection and Quarantine, the National Environmental Policy Act (NEPA), the Endangered Species Act (ESA), and the Texas Department of Agriculture, as follows:

1) Petition to TAG for resolution of conflicts of interest: 19 June 1989 (DeLoach 1989).
2) Reply from TAG, recommending approval: December 1991 (Cofrancesco 1991).
3) Petition to TAG (through state Departments of Agriculture of Texas, New Mexico, Colorado, Wyoming, Utah, Nevada and California for release of *Diorhabda elongata* into the field: 21 March 1994.
4) Approval from TAG 26 June 1995 (Cofrancesco 1995, Lehtonen 1995).
5) Biological Assessment to US-FWS, Region 2, Albuquerque, NM, 17 October 1997 (DeLoach and Tracy 1997).
6) Research Proposal, to US-FWS, Arlington, VA, 28 August 1998 (DeLoach and Gould 1998).
7) Letter of Concurrence, US-FWS, Arlington, VA, 28 December 1998 (Johnson 1998).
8) Environmental Assessment, published in Federal Register by USDA-APHIS, 18 March 1999 (Reed 1999).
9) Withdrawal of Letter of Concurrence, April 1999.
10) Revised Letter of Concurrence, US-FWS, Arlington, VA, 3 June 1999. Approved 10 release sites, one at Seymour, TX and 9 sites in CO, WY, UT, NV, CA, but eliminated all 5 proposed sites in the Pecos and Rio Grande Valleys (Laredo and Big Bend National Park, TX; and Artesia, Holloman AFB, and Bosque del Apache, NM and the site near Phoenix, AZ, and added the site requested at Delta, UT) (Frazer 1999).
11) FONSI, 7 July 1999 (Nave 1999).
12) Release permits from USDA-APHIS 12 July 1999, for Seymour, TX, and 9 sites in other states.

C. Clearances Needed for New Sites

A request to Region 2, U.S. Fish and Wildlife Service, Albuquerque was submitted on 14 February 2003 for 20 new release sites in 6 states, including 6 sites in Texas. The present proposal for releases on the upper Colorado River, at Lake Thomas TX with a subsite near Big Spring, TX was approved by Letter of Concurrence from FWS on 13 June 2003 for release of all *Diorhabda elongata* biotypes at Lake Thomas and Beal's Creek and Permits were received from APHIS-PPQ on 2 July 2003 for release of *Diorhabda elongata* biotypes from Crete and Turpan.

VI. MONITORING

Monitoring of the released beetles themselves and of the effects they produce on saltcedar and in riparian ecosystems is an absolute requirement of USDA-ARS and of the U.S. Fish and Wildlife Service for projects on biological control of weeds, at least during the research and demonstration phases. Monitoring will be performed in accordance with Monitoring Plans dated 7 April 1999. Monitoring will be performed by the biologist assigned to each site, assisted by a part-time employee, the latter both to accomplish the work and for safety in the field (both equipped with walkie-talkies and one with a cell phone). The biologist and helper will be trained, supervised, and assisted as needed and as time and resources permit, by the Biological Control of Weeds Team, at the USDA-ARS Grassland, Soil and Water Research Laboratory, Temple, TX and by the Texas A&M Research and Extension Center, Dallas, TX. Monitoring will measure the following:

A. The Beetles and Their Effects

1. Beetle development, survival and reproduction. The date of occurrence for each life stage (egg, larvae, pupae, adult) and number of eggs laid will be sampled periodically both inside and outside the cages once or twice weekly, with samples that estimate reproductive parameters and populations. Measurements of reproduction may be made by placing newly emerged pairs (male/female) of beetles in sleeve cages over healthy branch terminals and observing them twice weekly. Populations outside the cages are estimated by counting eggs, larvae (each instar), and adults present on 10 or more half-meter-long branch terminals on trees within the sample area.

2. Beetle Mortality factors. These factors are determined by observation of predators on sampled terminals. The major predators are expected to be spiders, ants, predaceous bugs (hemipterans), lady beetles and lace wings. Birds will be observed for predation on larvae and adults.

3. Beetle Overwintering. This is determined from observation of spring emergence in the 10X10 ft field cages.

4. Beetle Dispersal. This is measured by visual observations and sweep-net samples of eggs, larvae and adults taken along transects radiating outward from the release point. These samples are taken at the peak of each generation of last-instar larvae and of adults during the growing season.

5. Direct effects on saltcedar. Damage caused to the saltcedar plants will be made during sampling of beetle populations and dispersal (paragraph A1 and A4 above). Visual observations will estimate percent defoliation or foliage browning caused by beetle feeding, and length of dieback of branches and percent of branches affected on sampled trees.

6. <u>Effects on non-target plants</u>. Nearby non-saltcedar plants will be carefully observed during the dispersal measurements (paragraph A1 and A4 above). Observations include any adults, eggs or larvae present, observations of feeding, population of beetles present, and amount of damage. All plants will be observed, but especially *Frankenia* and athel (*Tamarix aphylla*) if any grow in the release area.

7. <u>Damage to saltcedar by other insects</u>. Observations will be made of the presence and populations of the beetles and of the type and amount of damaged caused by insects other than the *Diorhabda* beetles that attack saltcedar. The major other damaging insects are a leafhopper (*Opsius stactogalus*) and a scale insect (*Chianapsis etrusca*) that are host specific natural enemies of *Tamarix* in the Old World that were accidentally introduced along with *Tamarix* many years ago. Also, several native North American foliage-feeding and stem-boring insects occasionally damage saltcedar. The damage by all those insects can compound the effects of damage by the *Diorhabda* beetles and can mask the effect of the *Diorhabda* beetles.

B. Recovery of Native Vegetation Following Control

Monitoring of the native vegetation/saltcedar stands in the release area will be done according to the Monitoring Plan, attached herewith. This monitoring is done annually in late spring and/or late summer, beginning with the first year of the project to establish baseline conditions, and continues during each year of the project. This monitoring includes the measurement of growth parameters of 50 marked trees in concentric circles around the release point, and of characteristics of the native vegetation near the same marked trees. Annual remote sensing surveys will be conducted by low-level aerial photography at each site during late fall, when the color change of saltcedar makes it distinguishable from other vegetation. These surveys are expected to reveal the decrease in saltcedar stands and the increase in stands of native shrubs and trees as control progresses.

C. Recovery of Wildlife after Control

Monitoring of wildlife populations is conducted annually within the release area, beginning the first year to establish base-line data and continuing annually throughout the project. Monitoring should include birds and one other wildlife type such as butterflies, rodents or reptiles. The surveys are conducted to measure change in population over time as saltcedar is controlled and, if possible, between an area of near monotypic saltcedar and an area of near monotypic native vegetation. The bird surveys typically consist of 10 point counts (or area counts), in at each of 2 or 3 vegetation types (monotypic saltcedar, monotypic-native, mixed saltcedar and native) 5 minutes at each point, done three times during the breeding season each year. These surveys require highly skilled bird watchers that can identify the birds by both sight and song, possibly using paid volunteers from local Audubon clubs. The other types of surveys also require biologists skilled at identifying the types of wildlife surveyed. One skilled bird observer (with a second person for safety) usually can survey 10 point or area counts in one day, working from dawn to ca. 9:00 or 10:00 am. Thus, the total time required is 3 days (1 day per vegetation type) during each of 3 dates per year, times 3 years = 27 days.

VII. DELIVERABLES

<u>1. Quality Assurance Project Plan.</u> A QAPP must be submitted to EPA, through the TSSWCB, 60 days prior to the initiation of any sampling.

2. Quarterly Status Report. ARS will submit status reports on a quarterly basis.

3. Final Report. ARS will submit a final report upon completion of the project.

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VIII. Three Year Budget

	Federal	Non-Federal Match	Total
1. Personnel			
2. Fringe Benefits			
3. Travel			
4. Equipment			
5. Supplies			
6. Contractual	$99,246	$0	$99,246
7. Construction			
8. Other			
9. Total Direct Costs	$99,246	$0	$99,246
10. Indirect Costs			
11. Total Project Costs	$99,246	$0	$99,246

* See Budget Justification, next page.

Budget Justification

Technician Salaries and Fringe
Hire 2 part-time technicians, 6 mos. Each (24 wks) 960 hr @ $9.80/hr (GS-3)
One work March – August
One work June – November

Daily Tasks
4 days/week – conduct insect monitoring at Beal's Creek and Lake Thomas sites.
1 day/week – data entry, write weekly report
During May – June (9 days total) – assist bird monitoring person as needed
During June – assist vegetation monitors as needed for 4 days, complete vegetation monitoring if not completed by the monitoring team

Monitoring Required
Three types of monitoring of these sites is required by the U.S. Fish and Wildlife Service and by the USDA-APHIS-PPQ Release Permits: 1) control insect population, dispersal, and effect (control) of the saltcedar plants, 2) vegetation before, during and after control, and 3) wildlife before, during and after control. Monitoring is conducted according to the Monitoring Plan approved by FWS in 1999 and as modified April 2003 (attached).

Insect Monitoring
Each week, count numbers of adults, eggs, each instar larva, on 4 ea. 1-m branches and estimate total numbers per tree, measure size of tree, and estimate insect damage on each of the sample branches and on the entire tree, on 40 trees as described in the Monitoring Plan. At peak of each adult generation (ca. April 10, June 10, July 15, August 15, September 25) conduct surveys along 4 transects from the release point outward for 1 to 5 miles to detect beetle dispersion, population and damage to saltcedar. Monitoring will be conducted by personnel of the main project stationed at Temple, TX. Monitors will be assisted by the local technician employed under this grant.

Wildlife is monitored annually during the breeding season. Birds are monitored in the early morning at 10 point counts in each of monotypic saltcedar, pure native, and mixed saltcedar/native vegetation on 3 dates during May and June. Butterflies are monitored in the afternoons of the same days. Butterflies are monitored a second time during August. Per diem only is requested to cover the monitoring: $7,500/per year.

Vegetation is monitored once a year during June, using personnel from Temple and assisted by the local technicians employed under this grant. This monitoring requires ca. 2 ½ days for 5 persons working together, plus a half-day travel time at the beginning and end of the trip. Per diem only is requested under this grant: $7,500/per year.

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