Beginning success of biological control of saltcedars (Tamarix spp.) in the southwestern USA

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Summary
Saltcedars, Tamarix spp., exotic, invading deciduous shrubs or small trees from Asia and the Mediterranean area, have become the most damaging weeds of riparian areas in the western USA. We and our cooperators have obtained highly successful initial control of saltcedar by introducing the north Asian leaf beetle (Diorhabda sp., China/Kazakhstan ecotype) at five sites north of the 38th parallel, but they failed to establish farther south. In 2001, we discovered southern-adapted Diorhabda beetles on saltcedar and began testing them. In 2004, we released 2408 Greek beetles at Big Spring, TX; by September 2004, they had defoliated two trees, by 2005, 210 trees (0.8 ha) and by 2006, 7.3 ha of the saltcedar stand and 1.4 ha at Cache Creek, California, and had begun defoliating saltcedar at Pecos and Imperial, TX. The Uzbek beetles are increasing rapidly at Lake Meredith, TX and Fukang, China beetles at Artesia, NM, but the Greek and Tunisian beetles have not established near Kingsville in south Texas. We have revised the taxonomy of the five Tamarix-feeding Diorhabda ecotype/sibling species and predicted their climatic affinities in North America, correlated depletion of stored carbohydrates by beetle defoliation with plant death, developed pheromone attractants, remote sensing, improved release methods and a model of beetle dispersal and estimated possible damage to beneficial T. aphylla (Linnaeus) Karsten (athel) in the open field.

Keywords: saltcedar, athel, Tamarix, biological control weeds, Diorhabda.

Introduction
Western North American riparian ecosystems, beginning in the mid-1800s, have been invaded by exotic saltcedars (Tamaricales: Tamaricaceae: Tamarix spp.), shrubs or small trees native in Asia and the Mediterranean area (Baum, 1978). Three species have become serious weeds, Tamarix ramosissima Ledebour, Tamarix chinensis Loureiro and hybrids between them and other species in the western USA and northern Mexico and Tamarix parviflora de Candolle in California (Gaskin and Schaal, 2002, 2003). In addition, Tamarix canariensis Wildenow and Tamarix gallica Linnaeus (identification uncertain) and their hybrids with T. ramosissima and/or T. chinensis occur along the Gulf of Mexico coast from Louisiana to Port Isabel, TX but appear less invasive. Tamarix aphylla (Linnaeus) Karsten (athlon) is a large evergreen, cold-intolerant tree that

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is less invasive and is used for shade and windbreaks mostly in desert areas of northern Mexico and the southern third of the adjoining states in the United States; it is not presently a target for biological control. Saltcedars occupy approximately 800,000 ha of prime bottomlands from Montana into northern Mexico where they severely damage native plant and animal communities and rangeland ecosystems, including that of many endangered or threatened species (DeLoach et al., 2000).

Investigations into biological control began in 1986 by the US Department of Agriculture, Agricultural Research Service (USDA-ARS) at Temple, TX (DeLoach), and from 1991 with our overseas cooperators, R. Jashenko and I.D. Mityaev (Kazakhstan), R. Wang, Q.G. Lu, B.P. Li and H.Y. Chen (China), S. Myartseva (Turkmenistan), D. Gerling (Israel), A. Kirk, R. Sobhian and L. Fornasari (ARS, European Biological Control Laboratory (EBCL, France) and J. Kashefi (EBCL, Greece; DeLoach et al. 2004; Carruthers et al., 2008). Carruthers joined the project in 1998, followed by the other cooperators, especially after field releases began. This collaborative project includes scientists and their co-workers at each of the release sites and for specific research.

**Taxonomy and biology of Diorhabda spp.**

The first natural enemy chosen for introduction and testing in 1992 was the leaf beetle, *Diorhabda* sp. (Coleoptera: Chrysomelidae), from Fukang in northwestern China and Chilik in eastern Kazakhstan. In 2002, other more southern-occurring *Diorhabda* ecotypes were collected. All were identified by leading taxonomic experts as *Diorhabda elongata* (Brulé). Male and female genitalia were dissected from these collections and from some 700 museum specimens from over 370 locations in 37 countries of Asia and the Mediterranean area. J. Tracy (personal observation) found that the specimens fell into the five following ecotypes, corresponding to five probable areas of best adaptation in North America: (1) Fukang and Turpan, China and Chilik, Kazakhstan ecotype (deserts north of the 33rd northern parallel); (2) Crete and Posidi, Greece ecotype (Mediterranean climate CA, central TX); (3) Sfax, Tunisia ecotype (southern TX, southern coastal CA, Sonoran Desert, northern Mexico); (4) Karshi and Bukhara, Uzbekistan ecotype (plains grasslands of Oklahoma, New Mexico, Trans-Pecos, TX; Mojave Desert) and (5) coastal Iran ecotype (southern TX, Sonoran Desert), not yet imported.

Tracy’s species designations also are supported by other evidence. Cross-mating experiments that measured egg fertility [Thompson (with K. Gardner), New Mexico State University, Las Cruces (NMSU)], agreed with most of Tracy’s designations except that the beetles from Crete and Tunisia mated freely for several generations with little fertility decline and Karshi beetles hybridized with both Crete and Fukang beetles, albeit with reduced fitness in both crosses. DNA analyses supported species status for all five ecotypes examined (D. Kazmer, unpublished data). Pheromone analyses revealed that an aggregation pheromone produced by the males consisted of two complex alcohols, which varied in ratio between the four ecotypes from 1:1 to 1:30 (Cossé et al., 2005; A. Cossé, unpublished data). Differences in pheromones between species may partly explain why Tracy did not find hybrids among the many specimens examined from the Old World: The species with different pheromones were not attracted to each other and did not mate but may mate and produce hybrids when confined in cages. Both adults and larvae of *Diorhabda* feed and females oviposit on the foliage of *Tamarix*. The adults overwinter and the larvae pupate, under litter on the soil surface. The larvae have three instars and the life cycle requires approximately 34 days during summer. The beetles have two generations a year in areas north of the northern 38th parallel (Lewis et al., 2003b; Bean et al., 2007a,b) and three to five further south (Milbrath et al., 2007).

**Release, impact and establishment of the China/Kazakhstan ecotype of Diorhabda beetles**

The biological control program incurred a 6-year delay from the time we were prepared to release the *Diorhabda* beetles in June 1995 until actual release in May 2001. This was caused by the listing of the southwestern willow flycatcher, *Empidonax traillii extimus* Phillips (which had begun nesting in saltcedar in recent years, mostly in Arizona), as federally endangered (USDI-FWS, 1995). This required the submission of a biological assessment (DeLoach and Tracy, 1997) and consultation with the US Department of Interior-Fish and Wildlife Service (USDI-FWS). The resulting Letter of Concurrence allowed releases only at the ten specified sites in six states, only farther than 160 km from where the flycatcher nested in saltcedar. The first releases were to be in field cages for 1 year and extensively monitor beetle populations and dispersal, damage to saltcedar and any non-target plants and effects on native vegetation and wildlife communities. Populations of this flycatcher have increased greatly, especially at Elephant Butte Reservoir in central New Mexico and at Roosevelt Lake in central AZ after the mid-late 1990s, as willows increased after water level changes, to become among the largest breeding flycatcher populations known, nesting mostly in native willows (Sierra et al., 1997; Moore and Ahlers, 2005).

Overseas observations and quarantine testing at Temple, TX since 1992 and at Albany, CA since 1998 had demonstrated that these beetles were attracted to, fed and completed their life cycle only on *Tamarix* and
rarely on related species of *Frankenia* (order Tamaricales, family Frankeniaceae) on which the beetles probably cannot sustain a population in nature (Lewis *et al.*, 2003a; Dudley and Kazmer, 2005). These results led to agreements to release from FWS and the six state Departments of Agriculture and release permits from APHIS-PPQ.

These beetles were released in cages in 1999 and in the open field in May 2001 at ten sites in six western US states: California, Nevada, Utah, Colorado, Wyoming and Texas (DeLoach *et al.*, 2004). They established easily at five of the six sites north of the northern 38th parallel and, by 2006, had defoliated 30,000 ha of saltcedar at Lovelock, NV, 2000 ha at Schurz, NV Delta, UT, and Lovell, WY, and 150 ha (limited by surrounding herbicide and insecticide applications) at Pueblo, CO. However, these beetles did not establish at any of the four sites farther south in California or Texas or farther north in Montana or Oregon. At Lovelock, NV, 40% of the trees died where defoliation occurred twice annually for 4 years and 65% died where defoliated for 5 years (Carruthers *et al.*, 2008; T. Dudley, personal communication; J. Knight, personal communication), caused by gradual reduction in carbohydrate reserves (Hudgeons *et al.*, 2007). Tests by Lewis *et al.* (2003b) and Bean *et al.* (2007a) revealed that the lack of establishment in the southern areas was caused by a requirement of the beetles for 14.5-h summer-day length to avoid premature diapause. This day length occurs in the northern areas but is not reached south of the 38th parallel.

**Discovery, testing, release and impact of southern-adapted beetles**

Carruthers, with J. Kashefi (ARS-EBCL, Greece), discovered *Diorhabda* beetles on *Tamarix* in Crete in 2001. They were also found in Tunisia (A. Kirk, personal communication) and Uzbekistan (R. Sobhian, personal communication) in 2002. Testing by Bean *et al.* (2007b) revealed that all of these beetle populations were adapted to the southern areas of California, New Mexico and Texas where day lengths are shorter. Quarantine tests at Temple (Milbrath and DeLoach, 2006a,b) and at Albany (Herr, unpublished data) revealed that all are host-specific to *Tamarix* as is the Fukang/Chilik ecotype. Approvals were obtained from FWS, Texas, New Mexico and California Departments of Agriculture and APHIS-PPQ, and releases began in 2004.

**Releases, establishment and impact through 2007**

The first southern-adapted beetles to establish (from Crete) were released by DeLoach (with J. Tracy and T. Robbins) near Big Spring, TX. In April 2004, they released 37 adults and another 171 in July, that with their offspring, defoliated two small trees near the nursery cage in mid-July. Then, they released another 2200 adults in August that together defoliated a large tree near the cage by October 2004. By October 2005, the beetles had defoliated 210 trees covering 0.8 ha, by July 2006, 2.4 ha, and by October 2006, 10 ha. By October 2007, the beetles had dispersed 8.5 km and defoliated trees for 7 km along nearby Beals Creek. Remote sensing by low-level, aerial color photography by Everitt *et al.* (2007) clearly shows beetle-defoliated areas, permitting estimates of the areas of canopy that have been defoliated.

A mathematical model is under development to describe the advancing wave-form of beetle dispersal during each generation (J. Sanabria, personal communication).

The Crete ecotype also was released at 20 other locations in the Colorado River watershed in west Texas during 2005 to 2007 as part of a study by Knutson and Muegge (with E. Bynum) to develop optimum release methods. The beetles appear to be established at some sites. Along the Pecos River, they released approximately 500 adults from a nursery cage near each of Pecos and Imperial, TX, in early August 2006; by fall 2007, the beetles had defoliated approximately 500 trees along 1 km of the river near Pecos and had dispersed along 400 m of the river near Imperial. The Crete beetles failed at Seymour and Kingsville, TX, and increased and then declined at Lake Meredith, TX, and Artesia, NM. Crete beetles released in 2004 at Cache Creek (near Rumsey, CA) by Carruthers and Herr (unpublished data) established on *T. parviflora* in that area, increased rapidly during 2006, and by October 2007 had defoliated nearly all saltcedar for 32 km along the creek.

In the meantime, Michels (with V. Carney and E. Jones) released the *Diorhabda* ecotype from Posidi Beach (near Thessaloniki), Greece at Lake Meredith, TX, where they increased initially but then declined. The Uzbek ecotype, also released at this site, is increasing. Releases of Posidi beetles near Carlsbad, NM by O’Meara, and the Turpan China ecotype in southeastern Colorado by Eberts failed, probably because the sites flooded. The Fukang beetles released at Artesia, NM by Thompson (with K. Gardner) have adapted to shorter day lengths and appear to have established. Tunisian beetles were released in separate areas from the Crete beetles by Moran near Kingsville; both Crete and Tunisia beetles reproduced well in field nursery cages and in sleeve bags but failed to establish in the open field at this site.

**Possible causes of failure to establish**

The strong influence of short summer day length on induced premature diapause and failure to overwinter in southern areas was demonstrated by Bean *et al.* (2007a,b) with the Fukang/Chilik ecotype. In addition, distributional patterns of *Diorhabda* appear to be influenced by biome differences (e.g. desert versus maritime and continental versus maritime climates), latitude and elevation (J. Tracy, personal communication). Also important is the selection of sites unlikely to flood when
pupae or overwintering adults are on the soil surface and, of course, that will not receive herbicide or insecticide applications, mechanical controls or be burned or harmed by human disturbance. Attack on the beetles by arthropod predators in the trees or by ground beetles, mice, etc. on the ground may have caused some failures, which might be improved by selecting sites with simple vegetation communities that produce fewer insects that attract predators. At some locations, the rapid rate of increase of the Diorhabda beetles such as at Cache Creek, California, has allowed them to overcome predator attack. In addition, numbers released (which may influence production of sufficient concentrations of the beetle aggregation pheromone), cage type, time of year and the match of Diorhabda ecotype/species and Tamarix species/hybrids present all seem important to successful establishment. Differences in pheromone concentrations could explain differences in reproduction in cages vs. field. At some sites, beetles confined in cages mated and larval populations developed well, but after being released into the open field, the adults dispersed widely and the pheromone concentration may have been too low to allow mate finding. If populations are high, the beetles often migrate in swarms, which may maintain the necessary pheromone levels.

**Releases: Rio Grande, western Texas/Mexico and non-target effects on athel**

The largest and most damaging stands of saltcedars are along the Colorado River of Colorado, Utah, Arizona, California, and Mexico and the Rio Grande of New Mexico, Texas, and Mexico. We refrained from releasing Diorhabda sp. along the US–Mexican border because of Mexican concerns, including the potential for beetle attack on non-target athel (T. aphylla), which the Diorhabda beetles had attacked to some extent in our earlier laboratory and field-cage tests (Lewis et al., 2003a; Milbrath and DeLoach, 2006a,b; Herr et al., unpublished data). However, in preparation for possible agreement with Mexico for the release of the beetles along the Rio Grande, DeLoach (unpublished data) conducted overwintering cage tests along the Rio Grande between Presidio and Candelaria in western Texas with the Crete, Tunisia and Uzbek Diorhabda ecotypes, beginning in November 2006. All populations overwintered, although survival of the Crete beetles was slightly higher. Unfortunately, we then discovered that the Tunisian and Uzbek ecotypes had hybridized with the Crete ecotype in the outdoor cage cultures maintained at Temple, TX, and we destroyed these beetles before release. Uncaged, open-field tests were conducted at two locations in Texas to compare Diorhabda host selection for and damage to saltcedar, athel and Frankenia. At Big Spring in 2005, approximately 1- to 1.5-m-tall potted athel and local saltcedar and 10-cm-tall Frankenia plants were transplanted together in ten 1-m-diameter plots into an old saltcedar stand (4-6 m tall) being defoliated by Crete beetles released the year before. At Kingsville, Moran released over 6000 Crete and Tunisia beetles from 2004 to 2006 into an isolated stand of saltcedar previously inter-planted with 25 athel trees (both 3–4 m tall) and also onto large (10–12 m tall) roadside athel trees located 40 to 60 km away with no saltcedar present (no beetles were previously present at either site). The saltcedar at Kingsville is a hybrid between T. canariensis, T. gallica, T. ramosissima or T. chinensis, to which the beetles were not strongly attracted in outdoor-cage tests at Temple (Milbrath and DeLoach, 2006 a,b). At Big Spring, the beetles attacked only the saltcedar transplants from June to late August, but then, when populations increased to high levels, they attacked both saltcedar and athel. In 2005, DeLoach et al. (unpublished data) counted a total of 1711 adults on the saltcedar test plants, 588 on athel (34.5% of the total adults counted) and four adults on Frankenia. They also counted 170 egg masses on saltcedar, 30 on athel (15.0% of the total) and none on Frankenia. At Kingsville, Moran (unpublished data) found that Crete and Tunisia beetles developed and reproduced in sleeve bags on both saltcedar and athel but laid two- to fourfold more eggs on saltcedar. When adults were released from the sleeve bags, they laid two- to tenfold more eggs and produced many more larvae on saltcedar than on athel within the first 2 weeks of release and never established on athel. However, the beetles did not establish on saltcedar at Kingsville either, probably because the T. canariensis hybrid present was less attractive to them. When several hundred adults were placed in sleeve bags on the roadside athel plants, they produced larvae that defoliated branches inside sleeve bags, but when released, they simply flew away, apparently in search of a more attractive saltcedar species or hybrid, and never established a population on athel. In similar open-field tests at Cache Creek, California, in 2007 (Herr and Carruthers, unpublished data), the Crete beetles defoliated potted *F. salina* plants placed among the resident *T. parviflora* stand during August when the beetles reached high populations and defoliated the *T. parviflora*, but the *F. salina* plants later recovered. These open-field tests confirmed the cage-test results of Lewis et al. (2003a), Milbrath and DeLoach (2006a,b) and Herr et al. (unpublished data) that the female beetle searching for an ovipositional host plant is the most highly selective life stage. Adult plant selection for alighting/feeding or larval development is less specific. These results suggest that, if athel grows within a saltcedar stand, adults will alight on athel, but females will deposit fewer eggs than on saltcedar, leading to smaller larval populations and less damage to athel than to saltcedar. In the absence of a choice, i.e. if athel is spatially isolated from saltcedar or occurs in the midst of a defoliated saltcedar stand, the beetles would be unlikely to establish populations on athel or to cause damage sufficient to interfere with its use as a shade or windbreak tree.
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We met three times with Mexican scientists and officials, and in June 2007, they agreed not to oppose our releases along the Rio Grande west Texas. Therefore, on 29 June 2007, we released the Crete beetles from seven overwintering cages on five private ranches. So far, increases in beetle populations have been slow and most sites have flooded, but the beetles have survived at most sites, and at three sites populations have increased to a few hundred or a few thousand near the cages, suggestive of establishment. In general, the establishment of the southern-adapted beetles in the field has had a lower rate of success and lower rates of increase and dispersal than that of the Fukang/Chilik ecotype released in the northern areas. However, damage to saltcedar still is sufficient to promise successful biological control.

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