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Open field host selection and behavior by tamarisk beetles (*Diorhabda* spp.) (Coleoptera: Chrysomelidae) in biological control of exotic saltcedars (*Tamarix* spp.) and risks to non-target athel (*T. aphylla*) and native *Frankenia* spp. [☆]

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ABSTRACT

Biological control of invasive saltcedars (*Tamarix* spp.) in the western U.S. by exotic tamarisk leaf beetles, *Diorhabda* spp., first released in 2001 after 15 years of development, has been successful. In Texas, beetles from Crete, Greece were first released in 2004 and are providing control. However, adults alight, feed and oviposit on athel (*Tamarix aphylla*), an evergreen tree used for shade and as a windbreak in the south-western U.S. and México, and occasionally feed on native *Frankenia* spp. plants. The ability of tamarisk beetles to establish on these potential field hosts was investigated in the field. In no-choice tests in bagged branches, beetle species from Crete and Sfax, Tunisia produced 30–45% as many egg masses and 40–60% as many larvae on athel as on saltcedar. In uncaged choice tests in south Texas, adult, egg mass and larval densities were 10-fold higher on saltcedar than on adjacent athel trees after 2 weeks, and damage by the beetles was 2- to 10-fold greater on saltcedar. At a site near Big Spring, in west-central Texas, adults, egg masses and 1st and 2nd instar larvae were 2- to 8-fold more abundant on saltcedar than on athel planted within a mature saltcedar stand being defoliated by Crete beetles, and beetles were 200-fold or less abundant or not found at all on *Frankenia*. At a site near Lovelock, Nevada, damage by beetles of a species collected from Fukang, China was 12–78% higher on saltcedar than on athel planted among mature saltcedar trees undergoing defoliation. The results demonstrate that 50–90% reduced oviposition on athel and beetle dispersal patterns within resident saltcedar limit the ability of *Diorhabda* spp. to establish populations and have impact on athel in the field.

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1. Introduction

1.1. Origin and impacts of saltcedar

Exotic saltcedars (*Tamarix* spp., Tamaricaceae: Tamaricales, known as *cedros salados* in México) from Eurasia and Africa (Baum, 1978; Crins, 1989), introduced to North America for erosion control and as ornamentals in the 1800s, have come to dominate many arid riparian habitats in western North America (Robinson, 1965; Baum, 1967; Friedman et al., 2005; Birken and Cooper, 2006), causing great ecological and economic damage (DeLoach et al., 2000, in press; Zavaleta, 2000) to water and wildlife resources (van Hylck-

ama, 1980; Gay and Hartman, 1982; Weeks et al., 1987; DeLoach and Tracy, 1997; DiTomaso, 1998; Lovich and de Govenain, 1998; Kennedy and Hobbie, 2004; Shafroth et al., 2005). Saltcedar trees support reduced levels of faunal diversity compared to native vegetation (Boer and Schmidly, 1977; Engle-Wilson and Ohmart, 1978; Kerpez and Smith, 1987; Knutson et al., 2003). The two most widespread species, ranging from the northern Great Plains of the U.S. to northern México, are *Tamarix ramosissima* Ledebour and *Tamarix chinensis* Loureiro, which often form hybrids with each other and two other species, *Tamarix canariensis* Willdenow and *Tamarix gallica* L. (Gaskin and Schaal, 2003), which also occur in pure form along the Gulf coast of Texas and México (Crins, 1989; Gaskin and Schaal, 2003). A fifth species, *Tamarix parviflora* de Candolle, is most invasive in Pacific coastal drainages (Baum, 1967; Dudley et al., 2006). Saltcedar can be controlled by chemical herbicides (Sisneros, 1990; Duncan and McDaniel, 1998; Hart, 2006), mechanical removal, and burning (DiTomaso, 1998), but these methods are costly and can cause collateral damage to native plants and wildlife.

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1.2. *Athel* and *Frankenia* spp. plants related to saltcedar

Athel (*Tamarix aphylla* (L.) Karsten) (known as *pinabête* in México) is an exotic evergreen species of *Tamarix* that has been planted for shade and as a windbreak in urban and rural areas throughout the southwestern U.S. and northern Mexico (Baum, 1978; Crins, 1989; De Leon González and Vasquez Aldape, 1991), but its usage has decreased in recent years (DeLoach et al., 2008). Athel is invasive in Australia (Griffin et al., 1989), at Lake Mead and other sites along the lower Colorado River (Walker et al., 2006), along the Salton Sea in California (CA) (C.J.D., personal observation), and at sites in the Rio Grande Basin in Texas (TX) and Mexico (P.J.M. and C.J.D., unpublished data). Hybrids with *T. ramosissima* and *T. chinensis* have been discovered on Lake Mead in Nevada (NV), Blythe, CA, and along the Gila River east of Yuma, Arizona (AZ) (Gaskin and Shafroth, 2005). Nonetheless, athel is currently a nontarget plant in saltcedar biological control.

The genus *Frankenia* (family Frankeniaceae, Order Tamaricales) includes six native North American species, including *F. salina* (Molina) I.M. Johnston (alkali seaheath) in coastal and inland saline wet areas from central California to Baja California, western Sonora, Mexico and to Chile, and five uncommon, small desert shrubs: *F. palmeri* Watson in Baja California, western Sonora and southernmost California; *F. jamesii* Torrey ex. Gray from Colorado to western Texas; *F. johnstonii* Correll in southern Texas and north-eastern Mexico; and *F. gypsophila* I.M. Johnston and *F. margaritae* F. González Medrano in northern Mexico (Whalen 1987; Lewis et al., 2003a; Gaskin et al., 2004). Alkali heath provides food and habitat for wildlife; the wildlife values of the other *Frankenia* are unknown. These *Frankenia* do not occur in the same habitats as *Tamarix* but may occupy nearby habitats. Control methods targeting saltcedar must avoid or minimize impacts to these natives (Lewis et al., 2003a; DeLoach et al., in press).

1.3. Biological control of saltcedar

The potential for, research on, and results of the biological control program for saltcedar in North America have been extensively reviewed by DeLoach (1990, 2004), DeLoach and Tracy (1997), DiTomaso (1998), DeLoach et al. (2000, 2004, in press), Dudley et al. (2000), and Carruthers et al. (2008). The northern tamarisk beetle *Diorhabda carinulata* (Desbrochers) (Coleoptera: Chrysomelidae) (Tracy and Robbins, 2009), collected from Fukang, China and Chilik, Kazakhstan, was released at 10 sites in 6 western U.S. states (NV, UT, CO, WY, TX, and CA) beginning in May 2001, and have defoliated over 50,000 ha of saltcedar in Nevada and Utah (Geraci, 2006; DeLoach et al., 2007, in press), but enter diapause too early in southern latitudes (below 38°N), and do not overwinter or establish (Lewis et al., 2003b; Bean et al., 2007). Three species, collected from Uzbekistan (the larger tamarisk beetle, *Diorhabda carinata* (Faldermann)), Greece (Crete and Posidi) (the Mediterranean tamarisk beetle, *Diorhabda elongata* (Brullé)), and Tunisia (the subtropical tamarisk beetle, *Diorhabda sublineata* (Lucas)), are compatible with summer daylengths in Texas and other areas south of 38°N latitude (Milbrath et al., 2007; Tracy and Robbins, 2009). Mediterranean tamarisk beetles released in Texas in 2004–2005 have defoliated 6 ha of saltcedar along 9 km of riparian habitat and established satellite colonies over a 21-km area near Big Spring, in west-central TX (DeLoach et al., 2008, in press; Hudgeons et al., 2007a). Beetles of this species have also defoliated about 2.8 km of dense to scattered stands along the Pecos River, TX (Knutson and Muegge, 2008) and 500 acres along Cache Creek, near Rumsey, in north-central CA (Carruthers et al., 2008). Recent modeling results suggest that *D. sublineata* and *D. carinata* are most likely to establish under the daylength and climatic conditions prevalent in the Lower

Rio Grande Basin of Texas and northern México (Tracy and Robbins, 2009).

1.4. Host specificity testing of saltcedar beetles

Weed biological control agents often show oviposition and feeding preferences within the target host genus (Oickers, 2000; Medal et al., 2002; Sheldon and Creed, 2003). Extensive laboratory and outdoor cage testing demonstrated that the northern tamarisk beetle *D. carinulata* (known formerly as *D. elongata deserticola*) is completely restricted in host range to *Tamarix* (with only slight reproduction also on *Frankenia*); adults did not feed or oviposit, and all larvae died in the first instar, on all other plant species tested (Lewis et al. 2003a; DeLoach et al. 2003). The host specificities of *D. elongata*, *D. sublineata*, and *D. carinata* (known formerly as the Crete, Tunisia and Uzbek ecotypes, respectively, of *D. elongata*) were similar to that of *D. carinulata* in cage tests (Milbrath and DeLoach, 2006a,b). Across all four species, oviposition in multiple-choice tests in large (3 × 3 × 2 m) field cages (% eggs laid on each test plant) was 21–27% on *T. ramosissima* saltcedars, 20–30% on *T. parviflora*, 10–20% on *T. canariensis*/*T. gallica*, 9–11% on athel and 0–0.70% on *Frankenia*. Larval survival in outdoor sleeve bags at Temple was 48–82% on saltcedars, 43–100% on *T. parviflora*, 53–62% on *T. canariensis*/*T. gallica*, 34–75% on athel, and 2.2–17% on *Frankenia* (mean of three species). The reproductive index was 1.5- to 4.2-fold lower on athel than on saltcedar and at least 100-fold lower on *Frankenia* (Table 1). Similar outdoor cage tests also showed that *D. elongata* (Crete) beetles selected athel proportionately less as the degree of choice increased in large field cages, from 52.7% to 18.7% of eggs on athel in no choice vs. multiple-choice tests, and selection of athel was 84.2% lower than saltcedar in large (17 m³) cages but only 34.0% lower in small (1 m³) cages (Milbrath and DeLoach, 2006a,b). These results imply that further reductions in selection of athel should occur in open-field releases. The choice tests (Lewis et al., 2003a; Milbrath and DeLoach, 2006a,b) also showed that the most highly selective life stage is the ovipositing female. Adults (males and females) searching for a plant on which to alight and feed are less selective. The relatively high no-choice survival of beetle larvae (up to 100% of saltcedar in lab vials and bagged branches) on athel and *Frankenia* is not likely to be of consequence in nature because the females lay few eggs on these plants, resulting in low reproductive indices relative to saltcedar (Table 1). This difference should lessen the degree of impact on athel in the field. However, athel has not been evaluated as a host in the context of open-field releases of tamarisk beetles. Laboratory and field-cage based host preference results do not always accurately predict open field host range, due to the limitations of

Table 1
Reproductive index of *Diorhabda* beetle species/ecotypes on saltcedar, athel, and *Frankenia* spp.^a

Test plant	Beetle species			
	<i>Diorhabda elongata</i> (Crete, Greece)	<i>Diorhabda sublineata</i> (Tunisia)	<i>Diorhabda carinata</i> (Uzbekistan)	<i>Diorhabda carinulata</i> (Fukang, China)
Saltcedars				
<i>T. ramosissima</i> / <i>T. chinensis</i>	20.5	12.6	12.4	19.9
<i>T. canariensis</i> / <i>T. gallica</i>	11.9	8.1	5.6	13.8
<i>T. parviflora</i>	20.1	19.1	9.1	9.1
Athel (<i>T. aphylla</i>)	8.2	3.0	8.2	5.4
<i>Frankenia</i> spp.	0.07	0.00	0.103	0.006

^a Reproductive index calculated as percent larval survival in no-choice tests × percent oviposition in multiple-choice tests in cages. (DeLoach et al., in press; data from Milbrath and DeLoach, 2006b).

confined tests (Heard, 2000), and because climate, host quality, distances between hosts, predation and competition can influence field host ranges (Culliney, 2005; Sheppard et al., 2005). For example, the adventive leafhopper, *Opsius stactogalus* Fieber, can cause widespread damage to saltcedar foliage (Wiesenborn, 2003). In propagation cages and in the field (Big Spring, TX and Lovelock, NV), tamarisk beetles can exhibit ‘swarming’ behavior (P.J.M., C.J.D., and T.L.D, personal observation), which occurs when large numbers of beetles from an established population or overcrowded field cage disperse to nearby or distant saltcedar trees, and alight, feed, oviposit and establish on these plants.

1.5. Objectives of the study

We conducted uncaged, open-field tests to evaluate the degree to which *Diorhabda* tamarisk beetles differentiate between saltcedar and athel for alighting by adults and oviposition by females, and to compare damage inflicted on saltcedar and athel. We measured these characteristics using *D. elongata* (Crete) and *D. sublineata* (Tunisia) tamarisk beetles that were released into established stands of saltcedar interplanted with athel in south Texas. We also studied the behavior and ecology of *D. elongata* beetles invading resident saltcedar stands into which we had transplanted small saltcedar, athel and *Frankenia* plants in west-central Texas. Selection by *D. carinulata* (Fukang) beetles was also examined in Nevada. The ultimate objective was to identify the risks that southern-adapted *Diorhabda* beetles pose to athel, and also to *Frankenia*.

2. Materials and methods

2.1. Beetle collection, propagation, and biology

Southern-adapted *D. elongata* and *D. sublineata* tamarisk beetles (from Crete, Greece and Sfax, Tunisia, respectively) and *D. carinulata* (Fukang, China) were propagated from overseas collections, as described in Milbrath and DeLoach (2006a) and Tracy and Robbins (2009). Beetles were reared for at least one generation in quarantine at the Exotic and Invasive Weeds Research Unit, USDA-ARS, Albany, CA and were determined to be parasite- and pathogen-free. *D. carinulata* tamarisk beetles were released initially in 2001. *D. elongata* and *D. sublineata* tamarisk beetles were maintained on large saltcedar trees growing in outdoor cages at the USDA-ARS, Grassland, Soil and Water Research Laboratory, Temple, Texas and were shipped to field sites for further propagation in cages and releases in 2004 (Big Spring) and 2004–2006 (Kingsville). All collections were identified by two experts as ecotypes of *D. elongata* Brullé (Milbrath and DeLoach, 2006a). Subsequent morphological analyses by Tracy and Robbins (2009) indicated that four *Diorhabda* species have been released in North America. Mitochondrial (CO1) and nuclear (AFLP) DNA analyses (D. Kazmer, USDA-ARS, Sidney, MT, unpublished data), and examinations of endophallic sclerites in males (Tracy and Robbins, 2009), indicated that the ‘Tunisia ecotype’ beetles propagated successfully in cages and released in the 2006 no-choice and choice field tests at the south Texas sites were in fact *D. elongata* × *D. sublineata* hybrid beetles, generated inadvertently in outdoor cages at Temple, TX late in 2005. Similar hybrids generated via cross-mating (D. Thompson, New Mexico State University, Las Cruces, NM, unpublished data) were fully fertile for several generations in some backcrosses.

Both adults and larvae of *Diorhabda* spp. feed on the foliage of saltcedar. Females place from 1 to ca. 25 eggs (average 7–8) on the foliage singly or in tight clusters. In the laboratory at 28 °C (Lewis et al., 2003b; Milbrath et al., 2007), development requires 5.7 days for the egg stage, 13 days for the larval stages (three instars), and 5 days for the pupal stage (larvae drop from the foliage

into leaf litter for pupation), followed by a 6-day female preoviposition period and a 16-day oviposition period. The mean generation time is 39 days, and the average female produces 281 (*D. elongata*), 209 (*D. sublineata*), or 194 (*D. carinulata*) eggs. Population doubling time is 6.2 days. In the field, the life cycle requires about 30–35 days during the summer, with four generations in Texas below the 38th parallel (Milbrath et al., 2007; DeLoach et al., in press), as adults emerge from overwintering in late March–early May and exhibit overwintering/diapause behaviors beginning in mid-September to early November. Adults overwinter under litter on the soil surface or in clumps of grass.

2.2. Cages

The nursery cages used to produce beetles were 3.0 × 3.7 × 1.8–2.6 m, or 24.4 m³, made of 32-mesh/inch saran plastic screening over a 3/4 in. aluminum conduit frame, with a zippered door in one end. The bottom of the cage was buried to prevent the beetles from escaping. The sleeve bags used in all no-choice tests and to release beetles in the open-field choice test at Kingsville were made of 0.5 mm mesh, white polyester voil, 1 m long × 30 cm diameter. The bag was slipped over the terminal 1 m of a saltcedar or athel branch, the test beetles placed inside, and the open end tied around the stem.

2.3. Field site descriptions

2.3.1. Location of sites, climate and type of *Tamarix* present

Thirty-year normal winter temperature and precipitation varied between the three sites (Table 2). During the 2006 experimental period, daily minimum temperatures were higher and less variable and daily precipitation greater at Kingsville than at Big Spring (Table 2).

2.3.1.1. Kingsville and other south Texas sites. The Kingsville site is a 14 ha tract of private land not utilized for agriculture, but surrounded by croplands and pastures. It is located ca. 25 km south of Kingsville, and is 2 km from the north side of Baffin Bay, an inlet of the Laguna Madre adjacent to the Gulf of Mexico. The center is a low, saline, wet area of mixed saltcedar and native trees, shrubs, herbs and grasses, surrounded by 4 ha of sparse to dense saltcedar stands. The site contains a mixture of *Tamarix* species and hybrids, consisting of eight combinations of four haplotypes determined

Table 2

Physical characteristics and GPS locations of saltcedar and athel sites.^a

Temperature (°C) and precipitation (cm)	Site		
	Kingsville	Big Spring	Lovelock
30-Year averages^a			
Annual	22.0°	17.4°	10.4°
January daily min.	6.3°	−1.3°	−7.6°
July daily max.	35.3°	34.6°	33.2°
Annual precipitation	73.7° cm	51.1° cm	13.3° cm
2006 experiments^b			
Daily min.	21.8 ± 0.20°	16.8 ± 0.37°	ND
Daily max.	36.8 ± 0.25°	34.5 ± 0.33°	ND
Total precipitation	76.7° cm	16.1° cm	ND
GPS location			
Latitude	27.25°N	32.25°N	39.92°N
Longitude	97.48°W	101.39°W	118.75°W
Elevation	12 m	718 m	1208 m

^a Temperature and precipitation data from 1970 to 2000 climatic normals (NOAA, 2004).

^b Averages from 1 May 2006 to 30 September 2006 (±SE) from on-site continuous recorders; ND, not determined (see Fig. 2A for location of weather station at Big Spring).

from AFLP analysis of the *pepC* intron (Gaskin and Schaal, 2003; Gaskin and Shafroth 2005), including *T. ramosissima* × *T. chinensis*, *T. ramosissima* × (*T. canariensis* × *T. gallica*), *T. chinensis* × (*T. canariensis* × *T. gallica*), and two distinct *T. canariensis* × *T. gallica* haplotypes.

Two other south Texas sites were used in 2006 no-choice bagged branch tests, one near Sarita (27.11N, 97.48W) on U.S. Highway 77, 25 km south of the Kingsville site, and the other near Encino, TX (27.01N, 98.08W) on U.S. Highway 281, 63 km southwest of the Kingsville site. Both sites are roadside strips about 100 m long of large (10–12 m tall, trunks 20–30 cm diam. at 1.5 m height) athel trees with surrounding native and exotic grasses and forbs. No saltcedar is present.

2.3.1.2. Big Spring, TX site. The original release site is located on a private ranch 5–6 km east of Big Spring, Texas. The experimental site is about 22 ha flat floodplain beside Beals Creek on the east, bordered by gravelly hillsides 375 m to the west, near the release point (Fig. 1). Water seeps from the hillside after rains and can flood much of the site to a depth of 5–15 cm. The experimental area is located near the center of a larger *Diorhabda* beetle sampling area that extends approximately 11 km along Beals Creek. The resident saltcedar haplotypes at this site are predominantly pure *T. ramosissima* and *T. ramosissima* × *T. chinensis*, with lesser quantities of the *T. canariensis*/*T. gallica* haplotypes that are dominant in the Kingsville area.

2.3.1.3. Lovelock, NV site. This site is located about 10 km south of Lovelock in western Nevada and is in alkaline desert rangeland north of the Humboldt Sink. The site contains *T. ramosissima* and *T. ramosissima* × *T. chinensis* hybrids. The saltcedar infestation covers over 50,000 ha in this region. The 2007 study was conducted in the Humboldt Sink Wildlife Management Area (Nevada Department of Wildlife).

2.3.2. Plant communities at each site

The Kingsville, TX site is located in the Western Gulf of Mexico Coastal Grasslands ecoregion (Olson et al., 2001) and is dominated by honey – mesquite (*Prosopis glandulosa* Torr. var *glandulosa*) – Texas prickly pear (*Opuntia* spp.) – seaside ox-eye (*Borreria frutescens* (L.) DC) shrubland vegetation (Weakley et al., 1998). The Big Spring, TX site is in the Western Short Grasslands ecoregion (Olson et al., 2001), and consists primarily of saltgrass (*Distichlis spicata* (L.) Greene) and alkali sacaton (*Sporobolus airoides* (Torr.) Torr.) grassland and wetland bordering honey mesquite riparian grassland with intervening pickleweed (*Allenrolfea occidentalis* (S. Wats.) Kuntze) shrubland (Weakley et al., 1998). The site is bordered on the east by Beals Creek with dense mixed stands of large saltcedar, mesquite, and wolfberry (*Lycium* spp.). The Lovelock, NV site is in the Great Basin Shrub Steppe ecoregion (Olson et al., 2001) and is a sparsely-vegetated rangeland dominated by saltcedar and saltbushes (*Atriplex* spp.), greasewood (*Sarcobatus vermiculatus* (Hook) Torr.), and rubber rabbitbush (*Ericameria nauseosa* (Pallas ex Pursh) Nesom & Baird).

2.4. Saltcedar, athel, and *Frankenia* test plants used

2.4.1. Kingsville, TX

Resident saltcedar trees at the Kingsville site averaged 2.7 m tall with approximately 30 branches and had ≥95% green leaf material at the start of the 2005 and 2006 no-choice tests and the 2006 choice test. Athel saplings were propagated from 30-cm stem cuttings collected from mature trees near Mercedes, Texas, and grown in pots in a greenhouse maintained at 20–30 °C under partially-shaded natural light under an automatic mister until new shoots were ca. 15 cm long, then transferred to an outdoor slathouse

(50% shade), fertilized monthly and watered twice weekly. Stem cuttings (28 total) were placed in the ground in June 2003. All seedlings were located within a roughly elliptical 20 m × 50 m area among mature saltcedar. Plants were watered twice per week until the early spring of 2004. By the start of the 2006 open-field choice test, the athel trees were similar in size (2–3.5 m tall, 30–40 branches) to most of the saltcedar trees in the planting area.

2.4.2. Big Spring, TX

Resident saltcedar trees at the Big Spring site were 2–5 m tall with little or no *Diorhabda* defoliation at the beginning of each year but with 70–95% defoliation by late summer and fall during 2005 and 2006 tests, and about 30% regrowth during the spring of 2006. Saltcedar and athel transplants (1.0–1.5 m tall), propagated at Temple, TX (as above for Kingsville), and 20-cm tall × 30-cm diameter *F. salina* from Owens Valley, CA, *F. jamesii* from near Pueblo, Colorado (CO), and *F. johnstonii* from Zapata, TX were transplanted into 1-m diameter plots at the field site during May of 2005–2006 and watered weekly as needed.

2.4.3. Lovelock, NV

Resident saltcedar trees at the Lovelock site were 2–4 m tall and had been defoliated by Fukang beetles in 2005, with regrowth in 2006. Athel and saltcedar were collected in 2005 as 60–80-cm cuttings and grown for 10 months in 15-cm-wide pots, then transplanted into plots in April 2006 and 2007 with pot bottoms and sides cut away to allow water flow from the landscape. Plots were positioned 1–8 m from mature saltcedar trees.

2.5. Experimental design

During 2005 and 2006 at Big Spring, TX, tests were conducted in the open field by C.J.D. of host-plant selection of saltcedar and athel by adult saltcedar beetles for alighting and oviposition, and larval population development. At Kingsville TX, adult *D. elongata* and *D. sublineata* tamarisk beetles were placed in sleeve bags (P.J.M) for no-choice tests, and in 2006 hybrid *D. elongata* × *D. sublineata* tamarisk beetles were released into a stand of saltcedar interplanted with 25 athel saplings to conduct an open-field choice test, while attempting to establish a new beetle population. The opposite approach was taken at Big Spring (C.J.D.) and Lovelock, NV (T.L.D.), where athel, saltcedar and (Big Spring only) *Frankenia* spp. plants were transplanted into plots within saltcedar stands that were being defoliated by large, established populations of *D. elongata* (Big Spring) or *D. carinulata* beetles (Lovelock).

2.5.1. Kingsville tests: Mediterranean and subtropical tamarisk beetles

2.5.1.1. No-choice test. In June and July 2005, groups of 20 beetles (10 of each sex) were confined in sleeve bags for 1 week ($n = 10$ saltcedar and athel trees with *D. elongata* adults, $n = 5$ –7 trees per species with *D. sublineata* adults; one branch per tree). Eggs were then counted and all beetle life stages removed from the branches. In July 2006, groups of 20 *D. elongata* beetles were confined in bags on four large athel trees each at Encino and Sarita (two branches per tree) for 2 weeks. Hybrid *D. elongata* × *D. sublineata* beetles were confined on four athel trees in separate patches at each site. Beetles of each species or hybrid were also confined on two branches on each of four saltcedar trees at Kingsville. Egg masses and larvae (all three instars) were counted after 2 weeks and counts summed for each tree. Percent green and beetle-damaged leaf material on branches were visually estimated after 3 weeks.

2.5.1.2. Open-field choice test, 2006. On 8 June 2006, 20 hybrid tamarisk beetles (10 of each sex) were confined on each of five bagged branches on 10 saltcedar trees located within 5 m of at least one of 20 unbagged athel trees and 10 other unbagged

saltcedar trees selected for monitoring. Bags were removed after 2 weeks to release adults and relocate some larvae onto other branches of the bagged trees. Bags were re-secured for 1 week and then removed to release larvae for pupation.

Monitoring of bagged and unbagged saltcedar and athel began 2 weeks after adults were released, and was performed biweekly for a total of 12 weeks. On each of the 10 bagged saltcedar trees, three unbagged branches were selected for monitoring. Nine

Fig. 1. Layout of open-field saltcedar, athel, and *Frankenia* host selection tests along Beals Creek east of Big Spring, TX: (A) 2005 (21 September photo) and (B) 2006 (19 September photo). Reddish-brown trees are defoliated by *D. elongata* (Crete) beetles. TO, Transect origin (black lines) for the large-area monitoring program; NC, nursery cage; RT, original release tree; WS, weather station. (Aerial photos by James Everitt, USDA-ARS, Weslaco, TX.)

branches (three each in the lower, middle, and upper canopies) were selected on each of the 10 unbagged saltcedar and 20 athel trees. The total length of foliage on each branch was estimated by summing main branch length and that of all lateral branches ≥ 25 cm. On each monitoring date, counts of saltcedar beetle egg masses, larvae (all instars) and adults and predators (mainly unidentified spiders and assassin bugs (*Zelus tetracanthus* Stål, *Zelus renardii* Kolenati, and *Sinea* spp.) were made. Beetle eggs, larvae and adults were not removed. For the first 10 weeks, the percentages of branch length that was green, yellow, brown, or missing were visually estimated, as was percent beetle damage, distinguishable by its light brown color and by scarring on foliage and stems. Total branch damage (yellow, beetle and non-beetle brown, and missing foliage) was determined by summing. Counts of beetles and other arthropods were summed across all assessed branches within each tree and divided by total estimated green foliage length on assessed branches to yield densities per m green foliage length. To examine effects on foliage quality, estimates of percent damaged foliage were averaged across all monitored branches on each tree on each date.

2.5.2. Big Spring choice tests: established Mediterranean tamarisk beetles

2.5.2.1. Establishment of *Diorhabda* beetles in the open field. At Big Spring, on 22 April 2004, 37 adult *D. elongata* tamarisk beetles that had overwintered in an on-site nursery cage were released into the open field onto Tree RT (Fig. 1A). They moved to two small trees later designated as transect origin (TO) trees, produced many larvae, and by 15 July 2004 had defoliated these two trees. During July, another 171 adults were released and during August the remaining 2200 adults. By 3 August 2004, adults and larvae had begun defoliating large Tree #1, 25 m south of the TO trees (Fig. 1A), and by 3 October Tree #1 was 98% defoliated. By 21 October, beetles were found on 19 other trees, and a few nearby small trees were partially defoliated (Hudgeons et al. 2007). The beetles overwintered under litter and in large bunches of living or dead alkali sacaton grass near Tree 1, which we designated as the origin of dispersal (Fig. 1A). Beetles began emerging in April 2005, increased rapidly in population and dispersed through the first 20-m wide saltcedar thicket and the ‘near’ test plots by June (Fig. 1A), then across a 20-m wide grassy strip and about 20 m into a larger saltcedar thicket that contained the ‘far’ test plots, (Fig. 1A), defoliating about 1 ha (190 resident trees) by early October. During 2006, the beetle population increased rapidly in June. By early October 2006, they had reached high densities and had defoliated nearly all the saltcedar trees within about 10 ha of the study area (Fig. 1B), to 500 m northeast of Tree 1 and 300 m east to Beals Creek.

2.5.2.2. Location and establishment of plots. The first saltcedar–athel–*Frankenia* host selection experiment was carried out from May to October 2005, in areas where the beetles were dispersing and defoliating saltcedar, into the northeast quadrant from Tree 1 (Fig. 1A). In May of 2005, one 1.0 to 1.5 m-tall saltcedar, athel, and 20 cm-tall *F. salina* plant was transplanted into each of 10 circular 1-m diameter plots surrounded by ca. 5 × 10 cm mesh fencing to exclude livestock and wildlife. Pine bark mulch was placed around each plant and the plants were watered weekly as needed throughout the growing season. Five “near” plots were established 38–46 m from Tree 1 and five “far” plots 58–80 m from Tree 1 (Fig. 1A).

In 2006, the experiment continued outward along two transects (Fig. 1B), beginning at the outer edge of the 2005 defoliation front, with Transect A extending 350 m to the northeast and Transect B 200 m to the east of Beals Creek (Fig. 1B). Test plants, propagated as in 2005 were transplanted at stations 50–100 m apart, each with three fenced 1-m diameter plots 7 m apart, positioned randomly

within each station. One plot contained one saltcedar plant, one contained one athel (‘single’ plots), and one contained one athel and one saltcedar together, and also one *F. salina* and one *F. jamesii* or *F. johnstonii* (‘grouped’ plots).

2.5.2.3. Plot monitoring–2005. The plants were monitored on 15–16, 20 and 29 June; 5, 7–8 and 21 July; 25 and 31 August; and 7, 14–15 and 21 September. Average numbers were used for multiple counts in the same week, leaving 10 dates for presentation. Beetle populations and defoliation in the resident stand along Transect 2 (quadrats 1–6) (which ran through the test area) were examined along with the test plots (Fig. 1A). On each date, adults, eggs and larvae were counted and removed from the plots so that each count date would provide an independent population estimate. Count data for small (1st and 2nd instar) larvae were analyzed but not presented, as small larvae did not select a host plant, but remained on the plant on which they hatched. Large (3rd instar) larvae, did not have time to develop between observations, and arrived on test plants by crawling on the ground from resident saltcedar, but this test was not designed to measure larval plant selection.

In plot counts, persistent adults (those that apparently remained on the plants, increased gradually in population, and laid eggs) were separated from the large, transient ‘swarms’ (arbitrarily set at a minimum of 60, with up to 685 found on one plant, in 2006) that appeared suddenly, remained for one or a few days and rapidly defoliated the test plant, then apparently flew back into the resident saltcedar stand or dispersed out of the study area. When two ‘swarms’ occurred twice on the same plant during the same count week the average was listed. When one ‘swarm’ and one smaller persistent population occurred in 1 week, both were listed.

2.5.2.4. Plot monitoring–2006. Beetle populations and damage to saltcedar were monitored approximately weekly between 15 June and 10 October, a total of 18 weeks. Only seven adult beetles were counted on saltcedar at station 5 (250 m) and none at station 6 (350 m) on Transect A (Fig. 1B), because the beetles did not disperse that far, leaving eight stations for analysis. Monitoring was done as in the 2005 tests, except that individual eggs, and not egg masses, were counted, and counts of both small (1st and 2nd instar) and large (3rd instar) larvae were made. Beetle populations and progressive defoliation in the resident saltcedar stand along Transect 4 (quadrats 3–8) which ran through the test plots, were examined along with the test plots (Fig. 1B). Beetle ‘swarms’ were monitored as in 2005.

2.5.3. Lovelock choice tests: established northern tamarisk beetles

At the Lovelock site in 2006, six plots were positioned 1–8 m from the resident mature saltcedar plants. Each plot contained two *T. ramosissima* (collected from the Humboldt Sink, NV), two *T. parviflora* (collected from Cache Creek, CA), and two pure *T. aphylla* (collected from Lake Mead, NV). Five plots also contained two *T. aphylla* × *T. ramosissima* hybrids (from Lake Mead; hybrid status determined by Gaskin and Shafroth (2005)). Plants were 60–110 cm tall at the time of planting (12 May 2006). *D. carinata* tamarisk beetles were first observed on mature saltcedar trees on 1 July and larval densities reached 2500 per tree in 2006. Counts of adults, egg masses and larvae (1st and 2nd instars combined and 3rd instars) were made on 4 August 2006 and percent damaged foliage was estimated. Counts were summed across the two plants of each species/hybrid per plot. In a small additional test in 2007 to examine beetle damage, three plots, each containing one *T. ramosissima*, one *T. parviflora*, and one *T. aphylla*, were positioned 1–8 m from resident saltcedar trees, within the dispersal range of larvae crawling on the ground from these trees, and three

additional plots were set up 20 m from resident saltcedar trees, separated from them by a canal.

2.6. Statistical analyses

Beetle densities per m green foliage (Kingsville) or counts (Big Spring, Lovelock) (when summarized across all plot distances and types at Big Spring) from open-field choice tests are presented as least-square means of untransformed data in graphs (Figs. 2 and 6) and in Table 8. The Big Spring data are also presented as sums by date across plot distances and/or transects (or average of sums for multiple dates within 1 week) in Tables 4 and 6, and as sums per plant by distance and date (2005, Fig. 4) or sums across transects by distance and date (2006, Fig. 5). Foliage quality percentages from test trees at Kingsville and beetle damage at Lovelock are shown as means of untransformed data with 95% confidence intervals (Tables 3, 8 and Fig. 3). Defoliation percentages on resident saltcedar at Big Spring are given for informative purposes as means (Figs.

4G and 5I). All analyses used SAS software Version 9.1.3 (SAS Institute, 2005).

2.6.1. Kingsville field tests

Counts of beetle adults, egg masses and larvae from no-choice tests (Table 2) were ranked (ties = mean, SAS PROC RANK) and differences between plant species and beetle ecotype were examined with Kruskal–Wallis analyses of variance (Conover, 1998) using PROC GLM in SAS and least-significant difference (LSD) mean comparison ($P < 0.05$) (SAS Institute, 2005). For the choice test, the effects of tree type (bagged saltcedar release trees, unbagged saltcedar, or athel), time, and their interaction on ranked saltcedar beetle densities (per m green foliage) and on rank-converted percent green, damaged, and beetle-damaged foliage were examined using a multivariate analysis of variance with Wilk's lambda to determine significance ($P < 0.05$) and planned contrasts. The multivariate analysis was appropriate at Kingsville because beetles were not removed after counting, and so beetle densities at each of the six time points were not independent. When the tree type or time – tree type effect was significant, the effect of tree type was examined 2, 4, 6, 8, 10, and 12 weeks after bag removal using univariate ANOVA. Least-square means of ranks were compared across tree types with LSD tests.

2.6.2. Big Spring field choice tests

Analyses of covariance (Steel and Torrie, 1980) were performed involving plant species, date, distance group (distance along transect in 2006), and plot within distance group (or single/grouped plant plot type in 2006) as main effects, and plant $\times$ distance group and plant $\times$ time interactions in 2005 (distance $\times$ plant species $\times$ plant group type in 2006), with percent green foliage as the covariable (Tables 5 and 7). Other interaction terms were pooled into the experimental error term to add degrees of freedom to the error and increase statistical power. Least-square means of ranks derived from significant effects were adjusted by the covariable and compared between plant species with LSD tests. The covariate was not significant in analyses of small and large larvae in 2006 data and was therefore omitted. Transient large 'swarms' of adults, and of the eggs they laid are listed separately but were included in the analyses. Data for *Frankenia* spp. were not included in analyses, because only four adults and no eggs were found during 2005 and none of either in 2006.

2.6.3. Choice tests, Lovelock, Nevada

In the 2006 choice test at Lovelock, the effects of tree species (*T. ramosissima*, *T. parviflora*, *T. aphylla*, and *T. aphylla* $\times$ *T. ramosissima*) and distance from resident saltcedar on ranked beetle adult, egg, and larval counts) were examined 35 days after plot initiation (4 August 2006 and 16 July 2007) using analysis of covariance with green foliage as the covariate. Beetle damage after 35 days in 2006 and 2007 was assessed via ANOVA. The 35-day time interval was selected to evaluate ovipositional selection and impacts before defoliation of plot plants made comparisons difficult.

3. Results

3.1. Kingsville-no-choice field tests, 2005 and 2006

Diorhabda elongata and *D. sublineata* females deposited 3.9-fold and 2.1-fold more egg masses, respectively, on saltcedar than on athel after 1 week in 2005 at Kingsville, TX and this difference was significant for *D. elongata* (Table 3). *D. elongata* adults confined for 2 weeks on bagged branches in 2006 deposited 2.1-fold more eggs on saltcedar at Kingsville than on athel at Sarita and Encino,

Fig. 2. Densities of adults (A), egg masses (B) and total larvae (C) of *D. elongata* $\times$ *D. sublineata* hybrid beetles on unbagged branches on saltcedar trees from which beetles were released from bags (saltcedar-release), other saltcedar trees located within 5 m (saltcedar-unbagged), and athel trees within 5 m. Least-square means with differing letters are significantly different in Fisher's LSD tests on ranks ($P \leq 0.05$). Kingsville, TX, 2006.

Table 3

No-choice tests of oviposition by *Diorhabda* spp. adults from Crete or Tunisia confined for 1 or 2 weeks in 2005 and 2006 on bagged saltcedar and athel branches, and estimated percent foliage damage after 3 weeks.^a South Texas, 2005–2006.

Measure	<i>Diorhabda elongata</i> (Crete, Greece)				<i>Diorhabda sublineata</i> (Sfax, Tunisia) ^b				Statistical analysis ^c	
	n	Saltcedar	n	Athel	n	Saltcedar	n	Athel	F _{df}	P
Egg or larval counts (least-square mean $\pm$ SD) or percentage damage [(mean and (lower–upper 95% CI)]										
Eggs – 1 wk	10	21.2 $\pm$ 6.6 a	10	5.5 $\pm$ 6.6 b	7	15.4 $\pm$ 6.6 a	5	7.2 $\pm$ 6.6 ab	4.22 _{3,32}	0.013
Eggs – 2 wk	4	22.3 $\pm$ 6.2 a	8	10.4 $\pm$ 6.2 b	4	23.0 $\pm$ 6.2 a	6	13.3 $\pm$ 6.2 ab	6.31 _{3,18}	0.004
Larvae – 2 wk	4	146.8 $\pm$ 38.1 a	8	62.9 $\pm$ 38.1 b	4	181.2 $\pm$ 38.1 a	8	88.2 $\pm$ 38.1 b	10.7 _{3,20}	0.0002
Damage – 3 wk	4	87.5% ab (67.5–100)	8	79.9% b (67.9–92.0)	4	100% a	8	83.3% ab (63.7–100)	4.63 _{3,20}	0.013

^a Tests involved 20 beetles (10 male, 10 female) confined on 1-m branch lengths with 0.5-mm polyester mesh bags (1 m $\times$ 0.3 m) secured to the branch.

^b Counts after 2 weeks represent 2006 tests involving hybrid *D. elongata* $\times$ *D. sublineata* beetles.

^c F-values from Kruskal–Wallis tests on ranked data. Within each row, means followed by the same letter are not significantly different (Fisher's least-significant difference (LSD) test, $P < 0.05$).

while hybrid *D. elongata* $\times$ *D. sublineata* adults deposited similar numbers of eggs on saltcedar and athel over 2 weeks (Table 3). Total *D. elongata* and hybrid larval populations (all three instars) were 2.3- and 2.1-fold higher, respectively, on saltcedar than on athel after 2 weeks, both significant differences (Table 3). Beetles defoliated 90% or more of bagged saltcedar foliage and 80–90% of athel foliage (Table 3).

3.2. Kingsville field choice test, newly-released beetles, 2006

At Kingsville in 2006, hybrid *D. elongata* $\times$ *D. sublineata* tamarisk beetles confined on bagged branches for 2 weeks produced (mean $\pm$ SD) 350 $\pm$ 178 larvae per plant, and [mean (lower–upper 95% confidence interval) 99% (95–100%) of the green foliage inside the bags was killed by the larvae within 3 weeks of bagging. In a total of 1080 branch sampling events on athel and 720 events on saltcedar, 247 adults were encountered on the bagged and unbagged trees, 44% of which were on saltcedar; 252 total egg masses, 52% on saltcedar; and 1425 larvae, 46% on saltcedar. These raw counts did not take variation over time in green foliage availability into account. Twelve weeks after bag removal, 95.5% of athel branches ($n = 179$ monitored) and 98.3% of saltcedar branches ($n = 115$ monitored) had no saltcedar beetles of any stage, indicating that the beetles did not establish a population.

Multivariate time and time $\times$ tree type effects on adult beetle densities per m green foliage at Kingsville in 2006 were significant (Wilk's lambda, W), (time effect, $W = 0.166$, $F_{5,33} = 33.2$, $P < 0.0001$; time $\times$ tree type effect, $W = 0.345$, $F_{10,66} = 4.64$, $P < 0.0001$; tree type effect across all time points, $F_{2,37} = 0.27$, $P = 0.763$). Two weeks after release, adult densities were (mean $\pm$ SD) 0.16 ± 0.14 m⁻¹ on saltcedar release trees, 0.20 ± 0.40 m⁻¹ on unbagged saltcedar, and 0.006 ± 0.014 m⁻¹ on athel, a 27-fold difference between saltcedar and athel (Fig. 2A). Six weeks after release, overall adult densities (0.3 ± 0.8 m⁻¹) were the highest observed in the study, but did not differ statistically between saltcedar and athel (Fig. 2A).

Egg mass densities varied significantly by time ($W = 0.283$, $F_{5,33} = 16.7$, $P < 0.0001$) and time $\times$ tree type ($W = 0.373$, $F_{10,66} = 4.21$, $P = 0.0002$), although not by tree type across all time points ($F_{2,37} = 1.93$, $P = 0.159$). Egg mass density 2 weeks after release was 0.35 ± 0.36 m⁻¹ on saltcedar release trees, 0.21 ± 0.32 m⁻¹ on unbagged saltcedar, and 0 on athel (Fig. 2B). After 6 weeks, overall egg mass density was 0.30 ± 0.48 m⁻¹ on saltcedar release trees, 0.18 ± 0.20 on unbagged saltcedar, and 0.03 ± 0.05 on athel, a 10-fold significant difference between saltcedar release trees and athel (Fig. 2B). After 2 additional weeks, egg densities on unbagged saltcedar trees peaked at (0.21 ± 0.28 m⁻¹), differing significantly from the saltcedar release trees (0.10 ± 0.22 m⁻¹) but not from athel (Fig. 2B).

Larval densities varied significantly (time effect, $W = 0.118$, $F_{5,33} = 49.2$, $P < 0.0001$; time $\times$ tree type effect, $W = 0.355$, $F_{10,66} = 4.48$, $P < 0.0001$; tree type effect across all time points, $F_{2,37} = 0.64$, $P = 0.534$). Larval density 2 weeks after release was

Fig. 3. Percent green (A), total damaged (yellow, brown, and missing) (B), and beetle-damaged foliage (C) on unbagged branches on saltcedar trees from which *D. elongata* $\times$ *D. sublineata* hybrid beetles were released from bags (saltcedar-release), other saltcedar trees located within 5 m (saltcedar-unbagged), and athel trees within 5 m, 2–10 weeks after releasing adults. Bars indicate back-transformed means of arcsine-square root-transformed data + upper 95% confidence intervals. Means with the same letter at each time point are not significantly different in Fisher's LSD tests on ranks ($P < 0.05$). Kingsville, TX, 2006.

Fig. 4. Populations and dispersal of *D. elongata* (Crete) adults and eggs on saltcedar (A and B) vs. athel (C and D) expressed as counts per plant in plots. Distance in A–D is from Tree 1 (Fig. 1A). Graphs of beetles densities per m branch length (E and F) and percent defoliation (G) on resident saltcedar show distances along monitoring Transect 2 (Fig. 1A)). Big Spring, TX, 2005.

$1.8 \pm 3.6 \text{ m}^{-1}$ on saltcedar release trees, $0.42 \pm 0.41 \text{ m}^{-1}$ on unbagged saltcedar, and $0.004 \pm 0.01 \text{ m}^{-1}$ on athel, a difference of 100-fold between saltcedar and athel (Fig. 2C). Six weeks after release, overall larval density was $(1.0 \pm 1.1 \text{ m}^{-1})$, and averaged $1.1 \pm 0.72 \text{ m}^{-1}$ on release saltcedar, $0.95 \pm 1.1 \text{ m}^{-1}$ on unbagged saltcedar, and $0.45 \pm 0.52 \text{ m}^{-1}$ on athel, a 2.3-fold significant difference between saltcedar release trees and athel (Fig. 2C).

Percentage green foliage on saltcedar at Kingsville declined from 78–91% 2 weeks after release to 39–42% 10 weeks after release (Fig. 3A). Green foliage varied significantly over time ($W = 0.215$, $F_{4,34} = 31.1$, $P < 0.0001$) and in a time $\times$ tree type interaction ($W = 0.300$, $F_{8,68} = 4.88$, $P < 0.0001$). Athel branches maintained $>86\%$ green foliage (Fig. 3A) (tree type effect across all time points, $F_{2,37} = 23.1$, $P < 0.0001$; in two planned contrasts com-

paring athel and the two saltcedar tree types, $P \leq 0.0001$). The percentage of saltcedar foliage that was damaged (yellow, brown, or missing) increased to 58–60% by 10 weeks, while athel saplings showed no increase in damage (Fig. 3B) (time effect, $W = 0.212$, $F_{4,34} = 31.6$, $P < 0.0001$; time $\times$ tree type effect, $W = 0.292$, $F_{8,68} = 7.23$, $P < 0.0001$; tree type effect across all time points, $F_{2,37} = 23.8$, $P < 0.0001$; in two planned contrasts comparing athel and saltcedar, $P \leq 0.0001$). Damage characteristic of tamarisk beetle feeding comprised only 0–15% of total damage on saltcedar branches, but was significantly higher on saltcedar than on athel (Fig. 3C) (time effect, $W = 0.308$, $F_{4,34} = 19.1$, $P < 0.0001$; time $\times$ tree type effect, $W = 0.207$, $F_{8,68} = 10.2$, $P < 0.0001$; tree type effect across all time points, $F_{2,37} = 28.3$, $P < 0.0001$; in two planned contrasts comparing athel and saltcedar, $P \leq 0.006$). Six weeks after

Fig. 5. Populations and dispersal of *D. elongata* (Crete) adults, eggs and larvae on saltcedar (A–C) vs. athel (D–F), expressed as counts per plant summed across single- and grouped-plant plots and across test Transects A and B, for each station and distance. Distance in A–F is from the origin of station Transects A and B (Fig. 1B). Graphs of beetle densities per m branch length on resident saltcedar (G and H) and defoliation (I) show distances at quadrats along resident stand monitoring Transect 4 (Fig. 1B). Big Spring, TX, 2006.

release, beetle damage levels on saltcedar release trees (11%, 6.6–16.6) and unbagged saltcedar trees (6.9%, 4.4–10.1) were significantly higher than those on athel (1.0%, 0–3.4) (Fig. 3C). Foliage regrowth and declines in beetle density reduced beetle damage to ca. 3% by the tenth week after release (Fig. 3C).

3.3. Big Spring – field choice tests, established Mediterranean tamarisk beetles

3.3.1. Seasonal abundance of *Diorhabda* beetles

In 2005, overwintered *D. elongata* beetles from the 2004 releases (see Section 2.5.2.1) gradually dispersed out to 110 m along Transect 2 in the resident saltcedar stand (Fig. 1A) producing up to near 100% defoliation out to 90 m from Tree 1 after 15 September (Fig. 4E–G). Beetle populations on the test plants developed similarly, reaching high densities in late August. Populations of adults

and egg masses were lower on athel (Fig. 4C and D) than on saltcedar (Fig. 4A and B). On test plants (Table 4), populations began at low levels on saltcedar and athel in the five near plots, increased on saltcedar during the 2nd generation and reached high and defoliating densities on both plant species during the 4th generation by 31 August. Large ‘swarms’ of beetles were observed on three plants – 669 adults on one saltcedar in a ‘near’ plot on 7 September and 118 on each of two athel plants on 7 and 14 September (Table 4). Populations of eggs followed the same seasonal pattern but without ‘swarms’, with 4- to 15-fold lower levels on athel than saltcedar during the peak August–September period (Table 4). Populations of adults and eggs fell rapidly after mid-September (Fig. 4A and C), as the resident plants were defoliated (Fig. 4G).

In 2006, dispersal and defoliation continued from the 2005 area outward to 200 m along Transect 4 in the resident stand and along test plant Transects A and B to Beals Creek (Figs. 1B and 5G–I). The first generation from late May to late June was barely detectable in

Table 4Seasonal populations of *D. elongata* (Crete) beetle adults and eggs in an open-field test on saltcedar vs. athel. Big Spring, Texas, 2005.^a

Generation and week number	Date ^b	Adults ^a				Total adults	Egg masses ^a				Total eggs
		Saltcedar		Athel			Saltcedar		Athel		
		Near	Far	Near	Far		Near	Far	Near	Far	
<i>First</i>											
1	15–16 June	4	0	1	0	5	1	0	0	0	1
2	20 June	5	0	0	0	5	1	0	0	0	1
3	29 June	4	0	0	0	4	1	0	0	0	1
Total first		13	0	1	0	14	3	0	0	0	3
<i>Second</i>											
4	5, 7, 8 July	2	0	0	0	2	0	0	4	0	4
5	10–16 July	–	–	–	–	–	–	–	–	–	–
6	21 July	28	0	5	0	33	42	0	3	0	45
Total second		30	0	5	0	35	42	0	7	0	49
<i>Third</i>											
7–10	24 July–20 August	–	–	–	–	–	–	–	–	–	–
<i>Fourth</i>											
11	25 August	17	2	2	0	21	45	16	4	6	71
12	31 August	84	23	69	17	193	5	28	1	3	37
13	7 September	28 (+669 ^c)	28	57 (+118 ^c)	16	129 (+787)	13	2	0	2	17
14	14, 15 September (+swarms)	18	132 ^d	7	111 (+118 ^c)	268 (+118)	1	14	0	2	17
15	21 September	–	66	–	69	135	–	2	–	0	2
Total fourth (+swarms)		147 (+669)	251 0_	135 (+118)	213 (+118)	746 (+905)	64	62	5	13	144
<i>Total – all dates</i>											
Total near or far by plant		190	251	141	213	795	109	62	12	13	196
Total plant (%)		441 (55.5%)		354 (45.5%)		100%	171 (87.2%)		25 (12.8%)		100%
(+swarms)		(+669)		(+118)	(+118)	(+905)					
Total all SC or athel, near or far (% of total)		859 (50.5)	251 (14.8)	259 (15.2)	331 (19.5)	1700 (100%)	109 (55.6)	62 (31.6)	12 (6.1)	13 (6.6)	196 (100%)
Total on <i>Frankenia</i>		4 (0.23%)	0	0	0	4	0	0	0	0	0
Total beetles		1114 (65.4%)		590 (34.6%)		1704 (100%)	171 (87.2%)		25 (12.8%)		196 (100%)

Totals of rows and columns are in bold type.

^a Counts summed across five plots within each distance group; 'near' plots 1–5 are 38–46 m and 'far' plots 6–10 are 58–80 m from the origin of dispersal. See Table 5 for analysis of covariance, with % green foliage as the covariant. See Fig. 1A for plot layout, Fig. 4 for 3-D graphic display.^b Dates with 2 or 3 counts in the same week are presented as average per day (rounded to whole numbers). No counts were made between 8 and 15 July and between 11 July and 24 August.^c + numbers, apparent or incipient 'swarms' of 60 or more transient adults (see Section 2.5.2.3 for description of 'swarms').^d Not classified as a 'swarm' – 1, 10, 53, 14, 54 adults on 5 separate plants on same date.

Fig. 6. Counts of adults (A and B), eggs (C and D), small larvae (1st and 2nd instar) (E), and large larvae (3rd instar) (F) of *D. elongata* (Crete) per plant on each date, as influenced by plant species and plot groupings (single or grouped plants per plot; groupings summed for larvae). Counts averaged across transects and station distances. Graphs of least squares means are from analyses of covariance (see Table 7). Big Spring, TX, 2006.

test plots (Table 6 and Figs. 5A–F, 6), but the 2nd generation was many times larger, with 19–80 adults and 22–138 eggs counted each week across all plants in all plots (Table 6, and Figs. 5A–F, 6A–D), reflecting similar increases in the resident stand (Fig. 5G and H). The highest population densities in test plots were reached in the 3rd generation (Table 6); a large emergence of adults began on 23–25 August, and persistent adults reached a total of 132 per 4 plants on 6 September and 34 on athel on 1 September. From 14 to 30 August, we observed several ‘swarms’ of adults numbering up to 96 on individual saltcedar plants and to 685 on athel (Table 6), which, together with the persistent population, rapidly defoliated the affected test plants, as occurred in the resident saltcedar stand (Fig. 5G–I). Unlike 2005, in 2006 egg ‘swarms’, ranging from 60 to 247, were observed in the 3rd generation on saltcedar and 61 on athel (Table 6). Adult populations in the 4th generation remained high on saltcedar but not athel through 19 September (Table 6 and Fig. 6A, B), then decreased.

3.3.2. Open-field, uncaged choice test – 2005

3.3.2.1. Beetles on resident saltcedar. Within 4 months, the saltcedar beetles dispersed through two saltcedar patches encompassing all 10 experimental plots (Fig. 1A). By 21 September, saltcedar beetles had defoliated most of the resident saltcedar in the experimental area (Fig. 5G) as well as saltcedar and athel in the plots. In the meantime, regrowth had occurred in Plots #1–5 (Fig. 1A), and beetles defoliated these plots again by mid-September.

3.3.2.2. Beetles in test plots. During the 2005 season, a total of 1704 adults and 196 egg masses (including 3 adult ‘swarms’ on saltcedar) were counted across all plants and dates (90 sampling events) (Table 4). Adult beetles selected athel sevenfold less than saltcedar during the first two generations in ‘near’ plots positioned in a salt-

cedar thicket containing trees that were only 0–40% defoliated at the time, and no beetles alighted in the ‘far’ plots. During the much larger 4th generation they alighted and presumably fed on saltcedar twice as much as on athel across all plots (‘swarms’ included) (Table 4). Season-long adult populations of persistent adults were about equal on saltcedar (55.5%) and athel (44.5%). On 7 September, we saw the beginning of ‘swarming’ behavior by adults (see description of ‘swarms’ in Section 2.5.2.3) with 3 ‘swarms’ totaling 905 adults, which quickly defoliated these three plants. These ‘swarms’ shifted the population balance from nearly-equal numbers of persistent beetles to 65.4% on saltcedar and 34.6% on athel, with 4 (0.23%) found on *Frankenia*. In the near plots 1122 adults, 50.5% of the total were on saltcedar, 15.2% on athel and 0.23% on *Frankenia*. The far plots (582 adults), 14.8% were on saltcedar and 19.5% on athel (Table 4). Females deposited sixfold fewer eggs on athel than saltcedar during the first two small generations, and sevenfold fewer on athel during the 4th generation. Of the 196 egg masses (average 7.6 eggs per mass), 87.2% were on saltcedar, 12.8% on athel, and none on *F. salina* (Table 4). The ‘swarming’ adults laid no eggs on either test plant in 2005.

In analyses of covariance, which took into account the effect of defoliation, differences between plant species were highly significant for adults, eggs, and small larvae (Table 5). Differences between saltcedar and athel among sampling dates in adult ($P = 0.003$) (Table 5A) and egg mass counts ($P = 0.001$) (Table 5B) reflected four generational peaks, but also season-long spatial variation in adult beetle populations (Table 4 and Fig. 4A, C–F), as evidenced by the significant date, distance group, plots within distance group, and plant species $\times$ distance group interaction effects on adult counts (Table 5A). Saltcedar in the ‘near’ plots had more persistent adults than athel before these saltcedars were defoliated, and athel in the far plots had more adults than saltcedar

Table 5

Saltcedar vs. athel: analyses of covariance on ranked counts of *D. elongata* in experimental plots. Big Spring, Texas, 2005.

	df	Sum of squares	F	P-value
(A) Adults				
Plant species	1	28,000	8.90	0.003
Date	13	894,857	21.88	<0.001
Distance group (near/far)	1	59,967	19.06	<0.001
Plots within dist. group	4	33,885	2.69	0.032
Plant species × dist. group	1	18,764	5.96	0.016
Plant species × date	13	22,454	0.55	0.891
% Green foliage- covariable	1	113,722	36.14	<0.001
Error	194	610,435		
(B) Egg masses				
Plant species	1	44,857	10.99	0.001
Date	13	480,732	9.06	<0.001
Distance group (near/far)	1	40.2	0.01	0.921
Plots within dist. group	4	10,603	0.65	0.628
Plant species × dist. group	1	459	0.11	0.738
Plant species × date	13	75,900	1.43	0.148
% Green foliage –covariable	1	135,382	33.18	<0.001
Error	194	791,683		
(C) Small larvae				
Plant species	1	66,937	17.11	<0.001
Date	13	601,164	11.82	<0.001
Distance group (near/far)	1	15,257	3.90	0.050
Plots within dist. group	4	58,823	3.76	0.006
Plant species × dist. group	1	9236	2.36	0.126
Plant species × date	13	63,593	1.25	0.247
% Green foliage-covariable	1	92,110	23.55	<0.001
Error	194	758,877		

in the far plots in the 4th generation, after nearby resident saltcedars were defoliated (Table 4 and Figs. 4A, C, E, G). Both before and after defoliation, adults laid more eggs on saltcedar than on athel (Table 4 and Fig. 4B, D), explaining why distance and plot effects were not significant for eggs (Table 5B). Spatial variation in larval populations occurred, as larvae were more abundant in near than in far plots across both plant species (Table 5C).

3.3.3. Open-field, uncaged choice test – 2006

3.3.3.1. Beetles on resident saltcedar. During the spring of 2006, the overwintered adult population in the resident saltcedar stand was smaller than in the spring of 2005, but by mid-July the populations began increasing rapidly, and by early September they had colonized the plots to 200 m on both station transects (Fig. 5A–F) and had defoliated the resident saltcedar in almost the entire 10 ha study area (Figs. 1B, and 5G–I).

3.3.3.2. Adults in test plots. In 2006, of a total of 2420 adults, 49.5% were on saltcedar, 50.5% on athel, and none on *Frankenia* across all plots and 18 weekly counts (Table 6A). Of 1024 persistent adults, 69.9% were on saltcedar and 30.1% were on athel. In the single plant plots, 67.0% of the adults were on saltcedar and 33.0% on athel, while in the grouped plant plots 72.4% were on saltcedar and 27.6% on athel (Table 6A). When all four persistent beetle treatments were compared, 30.6%, 15.1%, 39.3%, and 15.0% were on single saltcedar or athel or on grouped saltcedar or athel, respectively (Table 6A). Alighting by persistent adults in single plots in comparison to grouped plots (on either saltcedar or athel) differed by only 8.6% (Table 6A).

Nine 'swarms' of 1396 adults were observed in the 3rd and 4th generations, seven from 18 August through 1 September, and two more through 19 September; 482 adults in five 'swarms' were on saltcedar (in both single and grouped plots) and 914 in four 'swarms' were on athel (three on single and one in grouped plots) (Table 6A). These 'swarms' substantially shifted the population bal-

ance, from twice as many persistent adults on saltcedar as on athel, to the opposite ratio (36.7%/63.3% saltcedar/athel) for the total (persistent + 'swarms') adult population in single plant plots, while adults were 50% more abundant on saltcedar than athel (74.0%/26.0%) in the grouped plant plots with 'swarms' included (Table 6A). Across all four plant species/plot type combinations, 24.1%, 41.6%, 25.4%, and 8.9% of the total adults were found on single saltcedar or athel or on grouped saltcedar or athel, respectively (Table 6A).

3.3.3.3. Eggs in test plots. In 2006, from a total of 1640 eggs counted, 73.1% were on saltcedar, 26.9% on athel and none on *Frankenia* (Table 6B). This included 954 eggs from persistent adults and 686 counted in association with 'swarming' adults. In all comparisons, about twice as many eggs were counted on saltcedar as on athel. Of the 954 eggs from persistent adults, 66.6% were found on saltcedar and 33.4% on athel (Table 6B). When all four treatments were compared individually for eggs from persistent adults, 29.6%, 13.1%, 37.0%, and 20.3% were counted on single saltcedar or athel or on grouped saltcedar or athel, respectively. Eggs associated with persistent adults differed by only 14.6% between single (42.7%) and grouped (57.3%) plant plots.

Eggs from adult 'swarms' were observed mostly between 23–30 August in the 3rd generation. The 1396 'swarm' adults laid 686 eggs (82.2% on saltcedar and 17.8% on athel) (Table 6B). These 'swarm' eggs increased the ratio of eggs from twofold to 2.7-fold (total eggs) between saltcedar (73.1%) and athel (26.9%) (Table 6B). Across the four plot type/plant combinations, 30.5%, 11.3%, 42.7%, and 15.5% of eggs were found on single saltcedar or athel or on grouped saltcedar or athel, respectively.

3.3.3.4. Season-long trends in 2006. In the analyses of covariance, both adults per plant ($P = 0.052$) (Table 7A, Figs. 5A, D, Fig. 6A, B) and eggs per plant ($P = 0.001$) (Table 7B, Fig. 5B, E, 6C and D) were significantly higher on saltcedar than on athel, with a similar trend for small (1st and 2nd instar) larvae ($P = 0.077$) (Table 7C, Figs. 5C, E, F), but no difference for large (3rd instar) larvae (Table 7D and Fig. 6F). Adult counts ('swarms' included) did not differ between plot groupings ($P = 0.120$, Table 7A), although the athel summed counts were only 8.9% of the total in grouped but 41.6% in the single-plant plots (Table 6A), while on saltcedar adults were only 13% higher in the grouped-plant plots (Table 6A), with the trend emerging most clearly late in the season (Fig. 6A, B). Eggs (from persistent and 'swarm' beetles) were 58.2% vs. 41.8% more abundant in grouped plots across both plant hosts (Table 6B), and eggs per plant were higher in grouped-plant plots ($P < 0.015$) for most of the 2006 season (Table 7B and Fig. 6C, D). Small larvae were also more abundant in grouped plots (Table 7C).

Station distance along transects influenced counts of adults, eggs, and small larvae in ANCOVAs (Table 7A–D), due to spatial and temporal heterogeneity in beetle populations in the plots (Fig. 5A–F) which, as in 2005 for adults and small larvae, was caused by the beetle population spreading outward, increasing in size, and advancing the defoliation front in resident saltcedar (Fig. 5G–I). The distance × plant species × plant grouping interaction in the ANCOVAs was significant only for adults ($P = 0.031$) (Table 7A).

3.4. Lovelock – field choice tests, established northern tamarisk beetles

At the Lovelock, Nevada site, a total of 23 *D. carinulata* adults were counted on all plants 35 days after plot initiation in 2006 (43% on saltcedar, 13% on pure athel). However, adults, eggs, and small (1st and 2nd instar) larval counts did not vary across the four hosts (Table 8). Third instars were 2.4-fold more abundant on saltcedar (37% of 343 counted) than on pure athel (16%), but did

Table 6Seasonal populations of *D. elongata* (Crete) adults and eggs in an, open-field test on saltcedar (SC) vs. athel, Big Spring, TX, 2006.^{a,b}

Generation and week		Single plants		Grouped plants		Plot groupings total		Test plant total	
Number	Date	SC	Athel	SC	Athel	Single	Group	SC	Athel
(A) Adults									
<i>First</i>									
1	15, 16 June	1	0	2	0	1	2	3	0
2	22 June	0	1	0	0	1	0	0	1
3	29 June	–	–	–	–	–	–	–	–
Total first		1	1	2	0	2	2	3	1
<i>Second</i>									
4	5 July	4	9	10	3	13	13	14	12
5	12, 14 July	2.5	3	9.5	4	5.5	13.5	12	7
6	17, 20 July	29.5	19.5	10	9	49	19	39.5	28.5
7	24, 27 July	13.5	9	15.5	15.5	22.5	31	29	24.5
8	31 July, 2 August	20	17	25	17.5	37	42.5	45	34.5
Total second		69.5	57.5	70	49	127	119	139.5	106.5
<i>Third</i>									
9	7, 9 August	17	5.5	8	4.5	22.5	12.5	25	10
10	14, 18 August	10	4.5 (+64)	30.5	8	14.5 (+64)	38.5	40.5	12.5 (+64)
11	23, 25 Aug	12.5 (+96)	7.5 (+103)	56 (+75)	6.5 (+62)	20 (+96, +103)	62.5 (+75, +62)	68.5 (+96, +75)	14 (+103, +62)
12	30 Aug, 1 Sep	17.5 (+81)	17.5 (+685)	45	16.5	35 (+81, +685)	61.5	62.5 (+81)	34 (+685)
13	6 September	64	4	68	25	68	93	132	29
Total third		121 (+177)	39 (+852)	207.5 (+75)	60.5 (+62)	160 (+1029)	268 (+137)	328.5 (+252)	99.5 (+914)
<i>Fourth</i>									
14	12 September	39 (+94)	15	69	22	54 (+94)	91	108 (+94)	37
15	19 September	83	30	49 (+136)	20	113	69 (+136)	132 (+136)	50
16	28 September	0	12	4	0	12	4	4	12
17	4 October	–	–	–	–	–	–	–	–
18	10 October	–	0	1	2	0	3	1	2
Total fourth		122 (+94)	57	123 (+136)	44	179 (+94)	167 (+136)	245 (+230)	101
<i>Totals, all dates</i>									
Persistent SC or Athel within plot grouping		313.5	154.5	402.5	153.5	468	556	716	308
% Plot group/each plant		67.0	33.0	72.4	27.6	45.7	54.3	69.9	30.1
Totals per plot grouping (%)		468 (45.7)		556 (54.3)		1024		1.024	
% Plant and plot grouping		30.6	15.1	39.3	15.0				
Total persistent beetles		1024 (100%)							
Swarms: total (no.)		+271 (3)	+852 (3)	+211 (2)	+62 (1)	+1123 (6)	+273 (3)	482 (5)	+914 (4)
Persistent + swarms		584.5	1006.5	613.5	215.5	1591	829	1198	1222
% of Total		36.7	63.3	74.0	26.0	65.7	34.3	49.5	50.5
Totals and %		1591 (65.7)		829 (34.3)					
% Plant and group		24.1	41.6	25.4	8.9				
Total all beetles		2420				2420		2420	
(B) Eggs									
<i>First</i>									
1	15, 16 June	0	0	57	29	0	86	57	29
2	22 June	0	0	0	0	0	0	0	0
3	29 June	–	–	–	–	–	–	–	–
Total first		0	0	57	29	0	86	57	29
<i>Second</i>									
4	5 July	23	0	4	0	23	4	27	0
5	12, 14 July	17.5	8	41.5	18	25.5	59.5	59	26
6	17, 20 July	34 (+72)	2	23.5	6.5	36 (+72)	30	57.5 (+72)	8.5
7	24, 27 July	9.5	2.5	10	0	12	10	19.5	2.5
8	31 July, 2 August	2	3	23	5.5	5	28.5	25	8.5
Total second		86 (+72)	15.5	102	30	101.5 (+72)	132	188 (+72)	45.5
<i>Third</i>									
9	7, 9 August	2.5	5.5	0	6.5	8	6.5	2.5	12
10	14–18 August	42	40 (+61)	47	32.5	82 (+61)	79.5	89	72.5 (+61)
11	23–25 August	44 (+85)	53	62.5 (+247, +100)	44 (+61)	97 (+85)	106.5 (+247, +100, +61)	106.5 (+247, +85 +100)	97 (+61)
12	30 August, 1 September	16 (+60)	5	29.5	16.5	21 (+60)	46	45.5 (+60)	21.5
13	6 September	5	6	16	24	11	40	21	30

Table 6 (continued)

Generation and week Number	Date	Single plants		Grouped plants		Plot groupings total		Test plant total	
		SC	Athel	SC	Athel	Single	Group	SC	Athel
Total third		109.5 (+145)	109.5 (+61)	155 (+347)	123.5 (61)	219 (+206)	278.5 (+408)	264.5 (+492)	233 (+122)
<i>Fourth</i>									
14	12 September	45	0	13	0	45	13	58	0
15	19 September	42	0	26	11	42	37	68	11
16	28 September	0	0	0	0	0	0	0	0
17	4 October	–	–	–	–	–	–	–	–
18	10 October	0	0	0	0	0	0	0	0
Total fourth		87	0	39	11	87	50	126	11
<i>Totals, all dates</i>									
Persistent SC or Athel within plot grouping		282.5	125	353	193.5	407.5	546.5	635.5	318.5
% Plot group/each plant		69.3	30.7	64.6	35.4	42.7	57.3	66.6	33.4
Totals per plot grouping (%)		407.5 (43.7)		546.5 (57.3)		954		954	
% Plant and plot grouping		29.6	13.1	37.0	20.3				
Total persistent beetles		954 (100%)							
Swarms: total (no.)		217 (3)	61 (1)	347 (2)	61 (1)	278 (4)	408 (3)	564 (5)	122 (2)
Persistent + swarms		499.5	186	700	254.5	685.5	954.5	1199.5	440.5
% of Total		72.9	27.1	73.3	26.7	41.8	58.2	73.1	26.9
Totals and %		685.5 (41.8)		954.5 (58.2)		1640		1640	
% Plant and group		30.5	11.3	42.7	15.5				
Total all beetles		1640 (100%)							

Totals of rows and columns are in bold type.

^a Saltcedar and athel plants (1–1.5 m tall) transplanted into a resident saltcedar stand being invaded by Crete beetles, at stations 1–4 along each of two transects, each station with three 1-m diameter plots, two with a single saltcedar or athel plant and one with both plants grouped in a plot. (See Fig. 1B for plot layout.)

^b Counts summed across all active stations on both transects on each week; multiple counts in the same week are averaged; “+” numbers are swarms or incipient swarms of 60 or more transient adults or eggs laid by them, with numbers of swarms in each column indicated in parentheses. See Fig. 6 for graphical display and Table 7 for covariant analysis.

not vary significantly across hosts (Table 8). Plant damage inflicted by tamarisk beetles was 11% greater on *T. ramosissima* saltcedar than on pure athel in 2006, a significant difference (Table 8), and was 53% greater on *T. ramosissima* [60.8% (22.8–98.9)] than on pure *T. aphylla* [7.5% (0.0–19.8)] after 35 days in the 2007 test ($F_{3,14} = 40.9$, $P < 0.001$) across both distances. Distance from resident saltcedar influenced damage in both years (for 2006, $F = 8.56$, $P = 0.009$; for 2007, $F = 69.4$, $P < 0.001$). In 2007, in plots located 20 m from resident saltcedar, only *T. ramosissima* received feeding damage [28.3% (9.4–47.3)]. Within 1 m of mature saltcedar, damage to *T. ramosissima* [93.9% (74.4–100)] was 78% greater than on athel [15.0% (0.0–47.8)], while *T. parviflora* had intermediate damage levels [38.3% (0–100)] at 1 m.

4. Discussion

4.1. Study sites

We conducted tests to measure selection by *Diorhabda* spp. tamarisk beetles for congeneric saltcedar and athel under natural conditions. The tests addressed concerns about damage to shade and windbreak athel trees arising from biological control of saltcedar along the Rio Grande in the U.S. and México. Hybrid *D. elongata* × *D. sublineata* and pure *D. elongata* tamarisk beetles distinguished mixed populations of *T. ramosissima*, *T. chinensis*, and *T. canariensis*/*T. gallica*, and hybrids of these saltcedar species from athel for adult alighting, oviposition and larval production in the field, and the same was true for damage caused by *D. carinulata*. However, the extent of prior beetle establishment on saltcedar, and the quality and diversity of choices offered in intermixed plantings (Kingsville) or test plots (Big Spring and Lovelock) influenced the resolution with which host choices by adult beetles could be detected.

The *Tamarix* species/hybrid mix occurring at the Kingsville, TX site contained many trees with at least some *T. canariensis*/*T. gallica* genetic content (Gaskin and Schaal, 2003), and these hybrids were only moderately acceptable to *D. elongata* and *D. sublineata* adults relative to *T. ramosissima*/*T. chinensis* species and hybrids in caged

choice tests at Temple, TX (DeLoach et al., in press) (see Section 1.4). The saltcedar blend at the Big Spring site was thus superior to that of Kingsville, and the resident saltcedar populations here and at the Lovelock, NV site were much larger. At Kingsville, *D. elongata* × *D. sublineata* hybrid tamarisk beetles did not establish a permanent population during the 2006 choice test, and the athel trees remained undamaged and survived several winters. At the Big Spring site, *D. elongata* beetles had already established a large population in resident saltcedar. They rapidly colonized and defoliated both resident trees and the relatively small saltcedar and, to a lesser extent, athel transplants in 2005 and 2006, and the athel saplings had to be re-planted each year due to unsuitable winter conditions. Plant resources available to beetles within the test plots and immediate surrounding area were thus more dynamic and at times more limited at Big Spring than at Kingsville, yet alighting and ovipositing adults showed a preference for saltcedar over athel at both sites. Resource limitations may have been greatest at the Lovelock site, at which the resident saltcedar trees had already been defoliated repeatedly prior to the test, thus forcing *D. carinulata* beetles to survive on limited regrowth on resident trees and on test plot plants, but even under these conditions the beetles damaged saltcedar more than athel.

4.2. Oviposition in no-choice tests

At the Kingsville and associated south Texas sites, *D. elongata*, *D. sublineata*, and hybrid tamarisk beetles confined on branches produced about twice as many egg masses and larvae on saltcedar than on athel, in broad agreement with prior choice tests in field cages (Milbrath and DeLoach, 2006a,b), but in contrast to prior no-choice tests in small field cages at Temple, TX, which found no differences in oviposition between saltcedar and athel (Milbrath and DeLoach 2006b; DeLoach et al., in press). In prior testing in outdoor cages, ovipositional host plant selectivity for saltcedar increased from no-choice to paired-plant choice to multiple-choice tests, (DeLoach et al., in press). Preference was somewhat reduced after 2 weeks of confinement at the Kingsville site compared to

Table 7

Saltcedar vs. athel: Analyses of covariance (A and B) or variance (C and D) examining effects of plant species on *D. elongata* beetle counts in plots, with plot grouping (single or grouped), sampling date and station distance as main effects and (for adults and eggs) foliage quality as the covariable. Big Spring, TX, 2006.

	df	Sum of squares	F	P value
(A) Adults				
Plant species	1	22,203	3.832	0.052
Date	22	702,586	5.512	<0.001
Distance along transect	2	35,013	3.022	0.051
Plant grouping in stations	1	14,097	2.433	0.120
Transect	1	406	0.070	0.792
Distance × plant species × plant grouping	4	63,056	2.721	0.031
% Green foliage – covariable	1	50,639	8.740	0.003
Error	213	1,234,085		
(B) Eggs				
Plant species	1	420,412	10.831	0.001
Date	22	305,849	3.582	<0.001
Distance along transect	2	32,690	4.211	0.016
Plant grouping in stations	1	23,254	5.991	0.015
Transect	1	4792	1.235	0.268
Dist. × plant species × plant grouping	4	203,206	1.190	0.210
% Green foliage – covariable	1	19,219	4.951	0.027
Error	213	826,759		
(C) Small larvae				
Plant species	1	15,392	3.167	0.077
Date	22	44,900	4.208	<0.001
Distance along transect	2	120,093	12.355	<0.001
Plant grouping in stations	1	21,612	4.447	0.036
Transect	1	1296	0.267	0.606
Distance × plant species × plant grouping	4	13,406	0.690	0.600
Error	206	1,001,189		
(D) Large larvae				
Plant species	1	3146	0.627	0.429
Date	22	365,741	3.311	<0.001
Distance along transect	2	84,719	8.438	<0.001
Plant grouping in stations	1	4370	0.871	0.352
Transect	1	5720	1.139	0.287
Distance × plant species × plant grouping	4	10,297	0.513	0.726
Error	206	1,034,180		

1 week, a likely artifact of resource depletion or limitations of the no-choice environment and their impacts on behavior (Heard, 2000; Sheppard et al., 2005). Prior results demonstrated 50–100% survival of larvae of *D. elongata* from Crete and *D. sublineata* from Tunisia on athel (Lewus et al., 2003a; Milbrath and DeLoach, 2006a,b), consistent with the 80–90% defoliation of both saltcedar and athel observed in the Kingsville no-choice tests.

Table 8

Open-field choice test of adult alighting and ovipositional selection by *D. carinulata* (Fukang, China) beetles on *T. ramosissima*, *T. ramosissima* × *T. aphylla*, pure *T. aphylla*, and *T. parviflora* in plots at Lovelock, Nevada in 2006.^a

Measure	Plant species				<i>F</i> _{df}	<i>P</i>
	<i>T. ramosissima</i>	<i>T. ramosissima</i> × <i>T. aphylla</i>	<i>T. aphylla</i>	<i>T. parviflora</i>		
Egg, larval or adult counts per plant, least-square means, (±SD) or percentage damage [(mean and (lower–upper 95% CI)] ^b						
Adults	1.6 ± 1.7 a	1.2 ± 1.7 a	0.7 ± 1.8 a	0.6 ± 1.6 a	0.56 _{5,17}	0.730
Egg masses	1.1 ± 1.1 a	0.8 ± 1.1 a	0.8 ± 1.2 a	0.5 ± 1.0 a	0.72 _{5,17}	0.619
Larvae – 1st and 2nd	3.1 ± 2.2 a	1.5 ± 2.1 a	3.0 ± 2.3 a	2.4 ± 2.0 a	3.95 _{5,17}	0.015
Larvae – 3rd	17.9 ± 5.3 a	13.6 ± 5.3 a	14.0 ± 5.6 a	13.9 ± 5.0 a	8.42 _{5,17}	0.0004
Damage	15.0% a (5.6–24.4)	16.0% a (5.8–26.2)	3.7% b (0.1–7.2)	7.2% ab (1.5–12.9)	8.43 _{4,18}	0.0005

^a Plots were monitored while Fukang beetles were defoliating regrowth on nearby mature saltcedar trees.

^b Counts summed across two plants per species within each plot and averaged across six plots (except five plots for the *T. aphylla* × *T. ramosissima* hybrid). *F*-values are from Kruskal–Wallis tests examining the influence of tree type on ranked beetle counts with percent green foliage as a covariable, or on ranked damage. Within each row, least-square means followed by the same letter are not significantly different (Fisher's LSD test, *P* < 0.05).

4.3. Field choice for saltcedar prior to establishment, Kingsville

Hybrid tamarisk beetles (*D. elongata* × *D. sublineata*) demonstrated a preference for saltcedar over athel for alighting and oviposition within 2 weeks, as reflected in 10-fold or higher egg, larval and adult densities on saltcedar than on athel. Adult densities peaked after an additional 4 weeks, indicating that a new adult generation emerged from larvae reared in bags, and these adults produced more egg masses and larvae on the saltcedar trees on which they were released than on athel, while not showing a preference for nearby unbagged saltcedar trees over athel. Aggregation pheromone releases by parental beetles (Cossé et al., 2005) could have influenced host selection by the new generation. The low overall beetle densities (0–1 beetle per m green length) and declines after 6 weeks were indicative of establishment failure at Kingsville, possibly caused by the somewhat lower suitability of *T. canariensis*/*T. gallica* saltcedar for oviposition by *D. sublineata* from Tunisia compared to other saltcedar species and hybrids (Milbrath and DeLoach, 2006b). The presence of athel did not itself contribute to failure, as larval survival and adult fecundity on athel and saltcedar do not differ (Milbrath and DeLoach, 2006a,b). Declines in green saltcedar foliage may help explain the failure of tamarisk beetles to establish at Kingsville. Both beetle and overall damage (inflicted mostly by *O. stactogalus* leafhoppers (Wiesenberg, 2003) were significantly higher on saltcedar than on athel. 'Spillover' effects (Blossey et al., 2001) on adjacent athel might have occurred if saltcedar trees had been defoliated by *D. elongata* and other herbivores. However, leafhoppers are prey items for assassin bugs such as *Z. tetracanthus* Stål, *Z. renardii* Kolenati, and *Sinea confusa* Caudell (Hemiptera: Reduviidae) (Bohmalk et al., 1996). Food web interactions (Pearson and Callaway, 2005) may have thus reduced *Diorhabda* densities on saltcedar, partially obscuring host preferences. Assassin bug predation on beetle larvae and adults was observed on 12 occasions at Kingsville in 2006 (P.J.M., unpublished data).

4.4. Field choice for saltcedar by established Mediterranean tamarisk beetles, Big Spring

In the 2005 and 2006 open field, uncaged tests near Big Spring, TX, 1-m tall potted athel and saltcedars were transplanted into strategic locations among a resident, mature saltcedar stand being invaded and defoliated by *D. elongata* tamarisk beetles. Beetles selected saltcedar over athel in test plots for alighting and oviposition in both years, but frequent (weekly) monitoring revealed the substantial influence of plot location, time, and both steady low density and episodic 'swarming' high-density beetle dispersal occurring before and during defoliation of resident trees.

4.4.1. Uncaged plot test in 2005

In 2005, the persistent population of *D. elongata* beetle adults selected saltcedar slightly more (55.5%) than athel (44.5%). However, when adult 'swarms' were included, selection for saltcedar (65.1%) was twice that for athel (34.6%). This result indicates that the adults did not search for athel in the 'far' plots (59–80 m from the 2004 dispersal point) until population pressures mounted and defoliation occurred in the 'near' plots and neighboring resident saltcedar thicket. *D. elongata* females were much less selective of athel (14.3% of saltcedar) in this test than in the several large outdoor multiple-choice tests at Temple, in which ovipositional selection for athel by the Crete collection of this beetle species was 43.8% as high as on saltcedar (Milbrath and DeLoach, 2006b).

4.4.2. Uncaged plot test in 2006

The 2006 plot test evaluated both plant host choice (saltcedar, athel, and two *Frankenia* spp.) and the influence of isolated vs. grouped saltcedar and athel on adult beetle host selection in the context of a dynamic *D. elongata* beetle population. As in 2005, ovipositing females selected saltcedar over athel (by 3.3-fold, eggs from persistent females only) in the first two generations, although adult selection for alighting was only 1.3-fold higher during the first 8 weeks of observation. During the 3rd generation, heavy (80–100%) defoliation of resident saltcedar and of test plants occurred, followed by a sudden sharp reduction in oviposition as the adults likely began fat storage in preparation for overwintering diapause, and/or responded to declining host quality/quantity in plots.

Opportunities for selectivity may have persisted longer into the field season in 2006 than 2005 because of the presence of grouped saltcedar and athel, which initially were selected slightly less often than the single plant plots for adult alighting, but which accumulated all of the 1st generation ovipositing adults, and later large numbers of both alighting and ovipositing adults from persistent generations and 'swarms' after August 1st. The increased biomass of saltcedar and athel plants together may have increased the attractiveness of grouped plots to large, mass-emerging and defoliating saltcedar beetle populations. Population estimates from the analysis of covariance exhibit peaks of great amplitude and short duration caused by the high reproduction rate, beetle aggregation ('swarming') behavior, and rapid defoliation of the test plants (often within 1 week), followed by migration to resident plants. In the 3rd and 4th generations in 2006, much higher peaks occurred in counts per plant adjusted by the defoliation covariant (Fig. 6) than in 'baseline' counts (Table 6), probably because of the concentration of the remaining adults, eggs and larvae on the small amount of remaining green foliage. The covariant analyses thus increased the apparent impact of large late-season beetle populations in test plots.

The trend toward higher numbers of small larvae on saltcedar reflects female oviposition preference, as these small larvae have little ability to select hosts. Late-season defoliation in plots was attributed in part to third instar larvae that arrived by crawling across the ground from damaged mature saltcedars and were likely not exposed to plant-derived behavioral stimuli or were insensitive.

4.4.3. Role of beetle 'swarms'

In both 2005 and 2006 we observed several large 'swarms' of adult beetles that fed and oviposited on test plants, which shifted the population balance in favor of one or the other test plants. In 2005, they altered a small preference for alighting on saltcedar to a 2-fold preference for saltcedar, but laid no eggs on either plant. In 2006, they erased a 2-fold preference of adults for alighting on saltcedar but strengthened the ovipositional preference for saltcedar from 2-fold to 3-fold. Swarms can have a major effect by defo-

liating a plant very quickly or by laying many eggs on it. This behavior, also observed in Kazakhstan and China, may represent a response to high populations and food shortages by providing a means of rapid dispersal to more distant undefoliated saltcedar trees, to enhance mate finding, and perhaps most importantly to inundate small areas of still low predator populations with sufficient beetles to overcome the predators; this behavior may be mediated by male aggregation pheromone that holds the swarm together (Cossé et al., 2005).

4.4.4. *Frankenia* selection in Big Spring plot tests

The 2005 and 2006 results for *Frankenia* spp. expand upon prior laboratory and field cage results (DeLoach et al., 2003; Lewis et al., 2003a), and two open-field tests with *D. carinulata* tamarisk beetles from Fukang (Dudley and Kazmer, 2005), as well as outdoor cage tests with *D. elongata* (from Crete), *D. sublineata* (from Tunisia) and *D. carinata* (from Uzbekistan) (Milbrath and DeLoach, 2006a). The past and present results together lead to the conclusion that the southern-adapted *Diorhabda* tamarisk beetles do not colonize or have negative impacts on *Frankenia* spp. in the field, with the possible exception of some transient damage to *F. salina* that grows in wet areas in the California/Baja California/Sonora area.

4.4.5. Biological control outcomes at Big Spring

By 2008, the *D. elongata* beetles had defoliated nearly all of 60 ha of saltcedar along 11 km of Beals Creek, often twice annually, with less regrowth each year, and now with about 25% saltcedar tree mortality and no feeding whatsoever on any other plants. After 3 years of defoliation, abundant local grasses and forbs had naturally revegetated the formerly bare soil. We expect that, with increasing and continuing control, native plant and wildlife diversity and populations will increase, stream flow here and reservoir levels downstream will increase, wildfires and surface salinities will be reduced, and streambank erosion, compared with other control methods, will be reduced because the saltcedar roots will die slowly and native vegetation will not be harmed.

4.5. Field choice for saltcedar by established northern tamarisk beetles, Lovelock

Reduced populations of *D. carinulata* on limited regrowth on repeatedly defoliated saltcedar, and increased predator densities (T.L.D., unpublished data) may have limited the ability of saltcedar at the Lovelock, NV site to support beetle populations to select hosts. Ovipositing *D. carinulata* (Fukang) adults did not discriminate between saltcedar and athel in 2006, in contrast to the Kingsville and Big Spring results. However, *D. carinulata* adults and larvae caused more damage on *T. ramosissima* saltcedar than on athel in plots in 2006 and 2007. Selectivity by the beetles at this site may have involved both adult and/or larval feeding components, although selectivity in caged tests relied on adult oviposition (DeLoach et al., 2003; Lewis et al., 2003a; Milbrath and DeLoach, 2006a,b). Differences in damage between *T. ramosissima* and *T. parviflora*, a host which was statistically equivalent to damage on athel in both years, could reflect reduced beetle preference for *T. parviflora* relative to *T. ramosissima*/*T. chinensis*, as shown in California cage and common garden tests (Dudley et al., 2006; Dalling et al., in press), although no differences in *D. carinulata* beetle alighting or oviposition were found between these saltcedar hosts in Texas cage tests (Lewis et al., 2003b; Milbrath and DeLoach, 2006a,b). Beetles inflicted more damage to athel in the presence (1 m plots) than in the absence (20 m plots) of adjacent resident saltcedar. The Lovelock results, similar to those at Big Spring, thus indicate that the degree of impact on athel is dependent on distance from resident saltcedar.

4.6. Implications for beetle establishment and impacts on athel

These results represent the first open-field evaluations of preferences of *Diorhabda* spp. beetles for saltcedar compared to athel. Our use of *D. elongata* and *D. sublineata* for these tests in Texas is appropriate because they are most likely to establish on saltcedars at the latitudes at which athel has been widely planted (Tracy and Robbins, 2009). The results demonstrate reduced oviposition and population development potential on athel relative to saltcedar when *Diorhabda* tamarisk beetles were dispersing in low numbers from mature saltcedar trees after release (Kingsville, Texas), or when defoliating mature trees at much higher densities (Big Spring, Texas), as well as reduced damage to athel when beetles were present at low densities in regrowth foliage (Lovelock, Nevada). Target-nontarget separation distance may be important in determining nontarget effects (Louda et al., 2005). In this study, distance between athel, resident saltcedar and the established beetle population influenced the results at Big Spring and Lovelock, with oviposition and damage to athel occurring mostly in the immediate proximity of a large saltcedar beetle population on resident saltcedar. The resulting defoliation impacts on athel saplings at Big Spring were exaggerated by their small size, relative to the mature athel trees that are widespread in the southwestern U.S. and northern México. Ongoing studies are addressing the question of whether southern-adapted *Diorhabda* spp. tamarisk beetles can establish on mature athel in the absence of a supporting saltcedar infestation, with results so far indicating a lack of establishment over two years (P.J.M., unpublished data).

Our results indicate that *Diorhabda* spp. tamarisk beetles are unlikely to establish or have long-term impacts on athel as a consequence of biological control of saltcedar, while not ruling out the possibility of transient but substantial damage to athel, as at the Big Spring site, by large adult populations and their progeny dispersing from defoliated saltcedar. As tamarisk beetles continue to be released in the Rio Grande Basin of Texas and México, we predict that they will cause no damage or only slight damage to athel, insufficient to impair their use for shade or as windbreaks, and these beetles will not establish independent, permanent and damaging populations on athel. The lack of tamarisk beetle establishment on athel and the potential invasive spread of athel over time will reduce or eliminate the potential conflict of interest posed by athel in saltcedar biological control.

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