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Biological Control 36 (2006) 32–48
Biological Control
www.elsevier.com/locate/ybcon

Host specificity of different populations of the leaf beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae), a biological control agent of saltcedar (*Tamarix* spp.) ☆

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Received 11 February 2005; accepted 16 September 2005
Available online 2 November 2005

Abstract

The leaf beetle, *Diorhabda elongata* (Brullé) *sensu lato*, was released in 2001 for the classical biological control of exotic saltcedars, a complex of invasive *Tamarix* species and hybrids. It did not establish at sites south of 37°N latitude where summer daylengths are below the critical photoperiod of the northern-adapted populations of the beetle that were released. Therefore, we assessed the host specificity of four *D. elongata* populations collected from more southern latitudes in the Old World (Tunisia, Crete, Uzbekistan, and Turpan, China). All populations were similar to each other and the previously released populations of *D. elongata* in their host specificity. Larval/pupal survival for all populations was 34–100% on *Tamarix* test plants, 0–76% on native *Frankenia* plants (both in the order Tamaricales), and 0% on the remaining 28 species of plants on which all the larvae died as 1st instars. *D. elongata* laid high numbers of eggs on saltcedar, generally fewer eggs on athel (a moderately valued evergreen species of *Tamarix*) except for Uzbekistan beetles, and few to no eggs on three species of *Frankenia*. Few to no adults were found on *Frankenia* plants which also were poor maintenance hosts. The release of any of the four *D. elongata* populations in the southern US and northern Mexico should pose no risk to plants outside the order Tamaricales and a low risk to native, non-target *Frankenia* plants. Athel may be less damaged than saltcedar.
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Keywords: Host range; Host specificity; Classical biological control; Weeds; Weed biological control; *Diorhabda elongata*; Saltcedar; *Tamarix*; *Frankenia*

1. Introduction

Saltcedars (*Tamarix* spp., Tamaricales: Tamaricaceae) are deciduous shrubs or small trees of riparian areas in deserts and steppes of Eurasia and Africa (Baum, 1978). Ten species of *Tamarix*, including nine different saltcedars and the single evergreen species *T. aphylla* (L.) Karsten (athel), were introduced into the United States and Mexico beginning in the early 1800s, primarily as ornamentals, for windbreaks and shade, and to stabilize stream banks (Baum, 1967; Crins, 1989; DiTomaso, 1998). Following the late 1920s, some of the saltcedar species became highly invasive along western riparian areas and lakeshores, with an early estimate of 600,000 ha of bottomlands infested (Robinson, 1965). The primary species involved in this invasion are *Tamarix ramosissima* Ledebour and *T. chinensis* Loureiro, as well as a common and widespread hybrid that has formed between these two species (Gaskin and Schaal, 2002). Additional invasive taxa include *T. parviflora* de Candolle, *T. canariensis* Willdenow, *T. gallica* L. (the latter two species being difficult to distinguish), and hybrids involving combinations of *T. ramosissima* and *T. chinensis* with *T. parviflora* and *T. canariensis*/*T. gallica* (Gaskin and Schaal, 2002, 2003). Saltcedar infestations currently range

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1049-9644/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2005.09.008

from North Dakota to Washington and south to northern Mexico. Both anthropogenic changes in western riparian ecosystems, which created ideal conditions for saltcedar invasion of disturbed areas, as well as the ability of saltcedar to invade and modify undisturbed environments, has created what is considered to be an ecological disaster for riparian areas of the West (DeLoach et al., 2000).

The *Diorhabda* leaf beetle from Fukang, China and Chilik, Kazakhstan, designated as *Diorhabda elongata* (Brullé) *deserticola* Chen by DeLoach et al. (2003) and by Lewis et al. (2003a,b), was released into the open field in 2001 in the United States for the classical biological control of saltcedar. Both adults and larvae defoliate saltcedar; 3rd instars are the most damaging stage (Lewis et al., 2003b). These beetle populations are established and increasing in abundance at most sites north of 37°N latitude (DeLoach et al., 2004). However, no establishment occurred at more southern sites where summer day lengths are less than 14 h 30 min, which is the critical photoperiod for the Fukang population (D.W. Bean, personal communication). In the southern areas, adult beetles enter reproductive diapause in early summer and presumably deplete their fat body reserves and starve before the following spring (Lewis et al., 2003b). This left large areas of some of the most serious saltcedar infestations, from Texas and Oklahoma west to southern California, without a biological control agent.

Other, more southern, populations of *D. elongata sensu lato* have shorter critical photoperiods for diapause induction (DeLoach et al., 2004; D.W. Bean, personal communication) and therefore are more likely to establish at more southern latitudes in North America. However, variation in this and other biological traits among populations of *D. elongata*, especially those distant from the original source of beetles released, may be accompanied by variation in their host range as well. A primary concern in the saltcedar biological control program has been assessing the risk posed to native, non-target plants in the genus *Frankenia* L., small shrubs of desert and salt marsh habitats (Lewis et al., 2003a). In North America, six species occur in the southwestern United States and northern Mexico (Whalen, 1980, 1987). The genus *Frankenia* is placed in the Frankeniaceae and together with the Old World family Tamaricaceae comprises the order Tamaricales (Spichiger and Savolainen, 1997). *Frankenia* spp. are the only native members of the Tamaricales found in North America (or even the Western Hemisphere); *Tamarix* is the only exotic genus of the order present (Whalen, 1980, 1987). A secondary concern involves the introduced athel, which is native to parts of southern Asia and northern and eastern Africa (Baum, 1978). It is a cold-intolerant, tree-sized, evergreen species of *Tamarix* that is grown as a drought-tolerant shade tree and windbreak, especially in northern Mexico.

We report here our evaluation of the host specificity of four populations of *D. elongata* collected across a wide geographical area of Eurasia and North Africa and below 43°N latitude, three of which had never been tested previously. This information, in combination with other biological studies of candidate populations, will provide the basis for determining the most promising population of *D. elongata* to release in the southern areas of the saltcedar infestation in North America.

2. Materials and methods

2.1. Insect colonies

The *Diorhabda* beetles collected on *Tamarix* in Asia and the Mediterranean area were all identified as *D. elongata* by A.S. Konstantinov (USDA-Agricultural Research Service Systematic Entomology Laboratory, Beltsville, MD) and/or I.K. Lopatin (Byelorussian University, Minsk, Belarus), although various names have been proposed in the literature (see DeLoach et al., 2003). Ongoing research by our team (J.L. Tracy, ARS, Temple, TX; D.J. Kazmer, J.F. Gaskin, ARS, Sidney, MT; D.W. Bean, J.C. Herr, ARS, Albany, CA; A.A. Cossé, R.J. Bartelt, ARS, Peoria, IL; and D.C. Thompson, New Mexico State University, Las Cruces, NM) indicates the probability of four species involved in our studies.

The four populations of *D. elongata* included in this study originated from North Africa to western China. They were collected 15 km south of Sfax, Tunisia (latitude 34.66 N, longitude 10.67 E, elevation 10 m); 3 km west of Sfakaki, Crete, Greece (latitude 35.83 N, longitude 24.6 E, elevation 7 m); 7 km west of Karshi (Qarshi), Uzbekistan (latitude 38.86 N, longitude 65.72 E, elevation 350 m); and at the Turpan Eremophyte Botanic Garden of Academia Sinica, ca. 10 km southeast of Turpan, Xinjiang Province, China (latitude 42.86 N, longitude 89.22 E, elevation 70 m below sea level). We here refer to these as *D. elongata* from Tunisia, Crete, Uzbekistan, and Turpan. Although the first host specificity tests in 1992–1993 involved *D. elongata* from or near Turpan, survival on all test plants in the initial larval no-choice test was very low and no oviposition occurred in the adult test that included native *Frankenia* plants (DeLoach et al., 2003). Therefore, in the present study we evaluated this population more rigorously. In addition, several tests involving *D. elongata* from Crete included a comparison with the previously released population from Fukang, China. Voucher specimens of *D. elongata* from all locations were deposited with the National Collection of Insects and Mites of the National Museum of Natural History, Smithsonian Institution, Washington, DC (under Lot Numbers GSWRL-2004-02 and -2005-02).

All beetles from overseas (except from Fukang) were brought into the USDA-ARS, Exotic and Invasive Weed Research Unit quarantine facility at Albany, California where parasites, predators, and pathogens were eliminated. Beetles (eggs and/or adults) were subsequently sent to the USDA-ARS Arthropod Containment Facility (quarantine) at Temple, Texas to initiate our own colonies or for immediate use in some tests. We obtained Fukang beetles from field colonies near Lovell, Wyoming in June 2002 from our cooperator D.J. Kazmer (USDA-ARS, Sidney, Montana).

The Fukang beetles had originally been cleared through the Temple quarantine facility. Beetle colonies at Temple were maintained either in the quarantine laboratory on potted *Tamarix* spp. (photoperiod of 16:8 h [L:D] and 28 °C [range 23–33 °C]) or in outdoor field cages on planted *Tamarix* spp.

2.2. Test plants

The test plants selected for host range testing of *D. elongata* were based on the revised list of DeLoach et al. (2003), which was developed according to the centrifugal phylogenetic method (Wapshere, 1974) using the angiosperm phylogeny of Spichiger and Savolainen (1997). Plants included US accessions of the saltcedars *T. ramosissima*, *T. chinensis*, *T. canariensis*/*T. gallica*, and *T. parviflora*, various saltcedar hybrids, athel (*T. aphylla*), three species of *Frankenia* (all in the order Tamaricales), and nine other related plants in the subclass Caryophyllidae (see Table 1 for names). Most *Tamarix* species and hybrids were identified by J.F. Gaskin (USDA-ARS, Sidney, MT) using the fourth intron of the nuclear phosphoenolpyruvate carboxylase (pepC) gene (Gaskin and Schaal, 2002). In addition, four unrelated habitat associates of saltcedar and 15 agricultural plants were included in the tests to have data available to answer concerns of landowners should they arise. As in the original tests, *Frankenia* spp. and athel were considered critical test plants, i.e., species within the order Tamaricales on which only a low level of damage was acceptable (DeLoach et al., 2003; Lewis et al., 2003a).

Plants were obtained from seeds, transplants or cuttings of plants growing in the field, or local nurseries. Some of the test plants had been planted on the Temple facility grounds. Seeds, transplants or cuttings were planted in 8-L pots containing a mixture of 10:3:2:1 parts of vermiculite, potting soil, peat moss, and sand. Cuttings and transplants were rooted under an automatic misting machine. Venus flytraps (*Dionaea muscipula* Ellis) were placed in 4-L pots containing a 1:1 mixture of peat moss and sand and were not fertilized. Agricultural test plants derived from seed were fertilized weekly with a 15-30-15 N-P-K soluble fertilizer. All other plants were fertilized twice a year with pellets of a slow-release fertilizer (15-9-12 N-P-K). Plants were held under natural daylengths and 24–35 °C in a greenhouse or natural temperatures in an outdoor slathouse prior to their use in tests.

2.3. Larval no-choice tests

We used a combination of laboratory tests utilizing excised foliage and field-cage tests with sleeve bags on potted test plants to determine the survival of *D. elongata* larvae offered a single type of test plant.

2.3.1. Vials, laboratory, 2002

We conducted a no-choice test of larval/pupal survival involving *D. elongata* from Crete and Fukang during June 2002 in the quarantine laboratory at 28 °C (range 25–32 °C) and a photoperiod of 16:8 h (L:D). The experimental design for each beetle population was a one-way treatment structure in a completely randomized design. Three replicates per test plant were used, which consisted of eight *Tamarix* species, accessions and hybrids, and three *Frankenia* species for the two populations. An additional nine species of related plants and four species of unrelated habitat associates of saltcedar were included for the Crete beetle (see Table 1 for names). Unfed neonate larvae (0- to 24-h old) were placed in separate 50 ml ventilated plastic vials. Each larva was provided excised leaves from a single type of test plant. Leaves were replaced every 2–3 days as needed and larvae or pupae were checked daily for survival and development. Between 10 and 13 larvae of each beetle population were provided each test plant species. Groups (replicates) of three to five larvae were used to calculate percentage survival to the adult stage.

2.3.2. Agricultural plants, vials, laboratory, 2003

Three series of larval no-choice tests were conducted in 2003. The first test evaluated the larval/pupal survival and development of *D. elongata* from Crete, Tunisia, Uzbekistan, or Turpan on 15 species of agricultural crops of importance to Texas and other states. These were: rice 'Cocodrie' (*Oryza sativa* L.), sorghum 'ATx2752*RTx430' (*Sorghum bicolor* (L.) Moench), winter wheat 'TAM III' (*Triticum aestivum* L.), corn 'CML325xTx732' (*Zea mays* L.), peanut 'Tamrun 96' (*Arachis hypogaea* L.), pecan (*Carya illinoinensis* (Wangenh.) K. Koch), watermelon 'Charleston Gray' (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), orange 'Valencia' (*Citrus sinensis* (L.) Osbeck), grapefruit 'Rio Red' (*Citrus x paradisi* Macfad.), cantaloupe 'Imperial 45' (*Cucumis melo* L.), soybean 'AG4701' (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), alfalfa (*Medicago sativa* L.), and grape 'Champanel' (*Vitis vinifera* L.). *Tamarix ramosissima* (Lovelock, NV; GenBank Accession No. AY090385) served as the control test plant. The test was done during June 2003 in the quarantine laboratory at 28 °C (range 25–31 °C) and a photoperiod of 16:8 h (L:D). The experimental design for each beetle population was a one-way treatment structure in a completely randomized design. Four replicates were used per test plant. Groups of four unfed neonate larvae (0- to 15-h old) from each of the four beetle populations were placed in separate 50 ml ventilated plastic vials and fed excised leaves from one of the test plant species. Leaves were replaced every 2–3 days as needed and the larvae or pupae were checked daily for survival and development. Sterilized sand was placed in the bottom of each vial when mature 3rd instars (final instar) were present to provide a pupation site. Surviving adults were counted upon emergence and percentage survival was calculated for each group of larvae.

2.3.3. Non-Tamaricales plants, vials, laboratory, 2003

The second no-choice test assessed the suitability of nine species of related plants (not including *Frankenia* species)

Table 1
Percentage survival (neonate to adult) of two populations of *D. elongata* on 11 Tamaricales and 13 other test plants: no-choice tests in vials, Temple, TX, June 2002[a]

Taxonomic grouping[b]	% survival (mean ± SD)	
	Crete, Greece	Fukang, China
Subclass Caryophyllidae, Polygonalian Lineage		
Order Tamaricales		
Family Tamaricaceae		
Tamarix aphylla[c,d] (L.) Karsten (athel) Uvalde, TX	75.0 ± 25.0 bc	60.0 ± 52.9 a
T. canariensis Willdenow/*T. gallica* L; Texas City, TX (AY090398, AY090437)	53.3 ± 5.8 cd	61.7 ± 12.6 a
T. chinensis Loureiro; Seymour, TX (AY090386)	85.0 ± 13.2 ab	63.3 ± 32.1 a
T. chinensis and/or *T. ramosissima* × *T. chinensis;* Artesia, NM (AY090386) and/or (AY090385, AY090386)	78.3 ± 20.2 abc	71.7 ± 30.1 a
T. parviflora de Candolle; Las Cruces, NM	100.0 ± 0.0 a	48.3 ± 27.5 ab
T. ramosissima Ledebour; Lovell, WY (AY090385, AY090396)	76.7 ± 2.9 bc	68.3 ± 16.1 a
Pueblo, CO (AY090385)	76.7 ± 25.2 ab	86.7 ± 23.1 a
T. ramosissima and/or *T. ramosissima* × *T. chinensis;* Bishop, CA (AY090385) and/or (AY090385, AY090386)	91.7 ± 14.4 ab	75.0 ± 25.0 a
Family Frankeniaceae		
[d]*Frankenia jamesii* Torrey; Pueblo, CO	13.3 ± 23.1 e	0 ± 0 b
[d]*F. johnstonii* Correll; Laredo, TX	6.7 ± 11.5 e	0 ± 0 b
F. salina[d,e] (Molina) I.M. Johnston; Point Isabel, CA	30.0 ± 8.7 d	6.7 ± 11.5 b
Order Nepenthales		
Family Droseraceae		
Dionaea muscipula Ellis (Venus flytrap)	0 ± 0 e	—
Order Plumbaginales		
Family Plumbaginaceae		
Limonium limbatum[e] Small (bordered sea lavender)	0 ± 0 e	—
Plumbago capensis Thundberg (blue plumbago)	0 ± 0 e	—
Order Polygonales		
Family Polygonaceae		
Rumex altissimus[e] Wood (smooth dock)	0 ± 0 e	—
Order Simmondsiales		
Family Simmondsiaceae		
Simmondsia chinensis (Link) Schneider (jojoba)	0 ± 0 e	—
Subclass Caryophyllidae, Caryophyllalian Lineage		
Order Caryophyllales		
Family Amaranthaceae		
Amaranthus blitoides Watson (prostrate pigweed)	0 ± 0 e	—
Family Chenopodiaceae		
Allenrolfia occidentalis[e] (Watson) Kuntze (pickleweed)	0 ± 0 e	—
Atriplex canescens[e] (Pursh) Nutall (4-winged saltbush)	0 ± 0 e	—
Family Portulacaceae		
Portulaca oleracea L. (purslane)	0 ± 0 e	—
Unrelated Habitat Associates		
Family Fabaceae		
Prosopis glandulosa[e] Torrey var. *glandulosa* (honey mesquite)	0 ± 0 e	—
Family Salicaceae		
Populus fremontii[e] Watson (Fremont cottonwood)	0 ± 0 e	—
Salix exigua[e] Nutall (coyote willow)	0 ± 0 e	—
Family Asteraceae		
Baccharis salicifolia[e] (R. & P.) Pers. (seepwillow baccharis)	0 ± 0 e	—

[a] $n = 3$ with 13 (Crete) or 10–11 (Fukang) larvae sampled per test plant. Values within each column followed by the same letter are not significantly different (Kruskal–Wallis Test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$).

[b] Taxonomic groupings follow angiosperm phylogeny of Spichiger and Savolainen (1997), and *Tamarix* follows the revision of Baum (1978) and the molecular identifications of test plant material by J.F. Gaskin (USDA-ARS, Sidney, MT). X denotes a hybrid. Accession numbers, if available, represent sequences of haplotypes of the pepC gene that are in the National Institute of Health's GenBank genetic sequence database. Accession numbers are given for each haplotype of heterozygous pepC genes, while a single accession number is given for the two identical haplotypes of homozygous pepC genes.

[c] Not confirmed by taxonomic authorities.

[d] Critical test plants.

[e] Habitat associates of *Tamarix*.

and four unrelated habitat associates of saltcedar (all outside the order Tamaricales, see Table 1 for names) for immature development and survival of *D. elongata* from Tunisia, Uzbekistan or Turpan. The control treatment, experimental design, and methods used were the same as described in the previous section.

2.3.4. Tamaricales, sleeve bags, outdoors, 2003

The third 2003 larval test utilized seven species and hybrids of *Tamarix* and three species of *Frankenia* that were fed to *D. elongata* larvae from Tunisia, Uzbekistan or Turpan (see Table 2 for names). Tests were conducted outdoors on the fenced grounds of the Temple ARS laboratory in $3 \times 3 \times 2$ m (length × width × height, 18 m^3) field cages at a mean minimum and maximum temperature of 22 and 36 °C, respectively, and a photoperiod of 16:8 h (L:D) (natural light supplemented with a halogen floodlight above each cage). The area immediately surrounding the field cages was treated periodically with hydramethylnon (tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone[3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethyenyl]-2-propenylidene]hydrazone, Amdro, Ambrands, Atlanta, Georgia) to control fire ant (*Solenopsis invicta* Buren) infestations. A permit authorizing testing in outdoor cages was received from USDA Animal and Plant Health Inspection Service in 1998 and was renewed in 2000. The experimental design for each beetle population was a one-way treatment structure in a completely randomized design, with five replicates per test plant. Groups of 10 neonate larvae (0- to 15-h old) from Turpan (July 2003), Tunisia or Uzbekistan (August 2003) were placed in individual 11×22 cm polyester organza sleeve bags that were securely tied to the branches of potted test plants. Two or three bags were used per plant. Plants were placed inside the large field cages and watered with a drip irrigation system. Surviving adults were counted upon emergence and percentage survival was calculated for each group of beetles. Some replicates were omitted from analysis due to ant predation.

2.4. Adult tests

We studied adult *D. elongata* host plant selection and oviposition preferences among different *Tamarix* and *Frankenia* species and hybrids using various paired- and multiple-choice tests. All other test plants, which did not support any larval development, were not included in these host specificity tests. We conducted all tests in outdoor cages on the fenced grounds of the Temple ARS laboratory. The primary focus of these tests was to investigate host acceptance by *D. elongata* of the native *Frankenia* species, in particular *F. jamesii* Torrey and *F. johnstonii* Correll. *F. salina* (Molina) I.M. Johnston also was included in some of these tests but was more fully assessed by the ARS laboratory in Albany, CA (J.C. Herr, unpublished data). A second focus involved the introduced athel. This plant was more fully tested in a separate study and additional results will be reported elsewhere.

2.4.1. Paired-choice test (Tamarix:Frankenia), small cages, outdoors, 2003

This test focused solely on adult colonization of and oviposition on the native species *F. jamesii* and *F. johnstonii* when paired with saltcedar by the various populations of *D. elongata* originating from Crete, Tunisia, Uzbekistan, and Turpan. Tests were conducted outdoors during July 2003 in $68 \times 53 \times 85$ cm (length × width × height, 0.3 m^3) aluminum screen cages placed inside $3 \times 3 \times 2$ m field cages, under a 16:8 h (L:D) photoperiod (natural light supplemented with a halogen floodlight above each field cage) and a mean minimum and maximum temperature of 22 and 38 °C, respectively. One potted saltcedar plant (*T. ramosissima* × *T. chinensis*, Pueblo, CO) and one potted *Frankenia* species (*F. jamesii* or

Table 2
Percentage survival (neonate to adult) of three populations of *D. elongata* on various *Tamarix* and *Frankenia* plants: no-choice tests in sleeve bags outdoors, Temple, TX, July and August 2003[a]

Test plants[b]	% survival [mean ± SD (*n*)]		
	Sfax, Tunisia	Karshi, Uzbekistan	Turpan, China
Tamarix parviflora Las Cruces, NM	66.0 ± 21.9 a (5)	50.0 ± 30.8 abc (5)	82.0 ± 21.7 a (5)
T. chinensis and/or *T. ramosissima* × *T. chinensis* Artesia, NM (AY090386) and/or (AY090385, AY090386)	65.0 ± 19.1 ab (4)	34.0 ± 27.9 bcd (5)	78.0 ± 20.5 a (5)
T. ramosissima × *T. chinensis* Pueblo, CO (AY090385, AY090386)	62.0 ± 14.8 ab (5)	45.0 ± 12.9 bcd (4)	82.0 ± 8.4 a (5)
T. canariensis/*T. gallica* Galveston, TX (AY090398, AY090437)	58.0 ± 11.0 ab (5)	58.0 ± 32.7 ab (5)	88.0 ± 8.4 a (5)
T. ramosissima and/or *T. ramosissima* × *T. chinensis* Bishop, CA (AY090385) and/or (AY090385, AY090386)	56.0 ± 18.2 ab (5)	66.7 ± 25.2 ab (3)	86.0 ± 8.9 a (5)
T. chinensis Pecos River/I-10, TX (AY090386)	52.0 ± 33.5 ab (5)	48.0 ± 17.9 abc (5)	78.0 ± 13.0 a (5)
T. aphylla[c] (athel) Encino, TX	34.0 ± 33.6 bc (5)	75.0 ± 19.1 a (4)	67.5 ± 12.6 a (4)
Frankenia jamesii[c] Pueblo, CO	0.0 ± 0.0 c (3)	0.0 ± 0.0 d (3)	54.0 ± 35.1 a (5)
F. johnstonii[c] Laredo, TX	7.5 ± 5.0 c (4)	18.0 ± 24.9 cd (5)	76.0 ± 8.9 a (5)
F. salina[c] Owens Valley, CA	10.0 ± 17.3 c (3)	26.0 ± 20.7 cd (5)	66.0 ± 21.9 a (5)

[a] Ten larvae were sampled per replicate of each beetle population. Values within each column followed by the same letter are not significantly different (Kruskal–Wallis Test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$).
[b] Molecular identifications of *Tamarix*, including GenBank accession numbers if available. X denotes a hybrid.
[c] Critical test plants.

Table 3
Presence of adult *D. elongata* from four different populations 1, 2, and 5 days post-release on *Tamarix* and *Frankenia* plants: paired-choice (*Tamarix*:*Frankenia*) tests in small cages outdoors, Temple, TX, July 2003[a]

Location of adults:	No. adults per location (mean ± SE)[b]				*P* value[c]
	Crete, Greece	Sfax, Tunisia	Karshi, Uzbekistan	Turpan, China	
	Tamarix ramosissima × *T. chinensis*[d] vs. *Frankenia jamesii*				
	1 day post-release				$P = 0.06$
T. ramosissima × *T. chinensis*	14.0 ± 1.6	10.2 ± 1.2	14.6 ± 1.2	9.8 ± 1.2	a
F. jamesii	0.0 ± 1.6	0.2 ± 1.2	0.0 ± 1.2	0.0 ± 1.2	d
Cage walls	1.3 ± 1.6	3.4 ± 1.2	1.8 ± 1.2	3.0 ± 1.2	c
Dead/unaccounted	4.7 ± 1.6	6.2 ± 1.2	3.6 ± 1.2	7.2 ± 1.2	b
	2 days post-release				$P = 0.52$
T. ramosissima × *T. chinensis*	14.3 ± 1.5	11.6 ± 1.2	14.0 ± 1.2	11.4 ± 1.2	a
F. jamesii	0.0 ± 1.5	0.2 ± 1.2	0.0 ± 1.2	0.0 ± 1.2	d
Cage walls	2.0 ± 1.5	2.0 ± 1.2	2.2 ± 1.2	2.4 ± 1.2	c
Dead/unaccounted	3.6 ± 1.5	6.2 ± 1.2	3.8 ± 1.2	6.2 ± 1.2	b
	5 days post-release				$P < 0.01$
T. ramosissima × *T. chinensis*	17.0 ± 1.2 a	13.2 ± 0.9 b	16.0 ± 0.9 a	11.2 ± 0.9 b	
F. jamesii	0.0 ± 1.2 e	0.6 ± 0.9 e	0.6 ± 0.9 e	0.2 ± 0.9 e	
Cage walls	1.0 ± 1.2 de	3.6 ± 0.9 cd	1.6 ± 0.9 de	3.8 ± 0.9 cd	
Dead/unaccounted	2.0 ± 1.2 cde	2.6 ± 0.9 cde	1.8 ± 0.9 de	4.8 ± 0.9 c	
	Tamarix ramosissima × *T. chinensis* vs. *Frankenia johnstonii*				
	1 day post-release				$P < 0.01$
T. ramosissima × *T. chinensis*	12.3 ± 1.5 a	13.8 ± 1.2 a	13.4 ± 1.2 a	6.6 ± 1.2 cd	
F. johnstonii	0.0 ± 1.5 fg	0.0 ± 1.2 g	0.0 ± 1.2 g	0.0 ± 1.2 g	
Cage walls	3.0 ± 1.5 defg	2.6 ± 1.2 efg	2.6 ± 1.2 efg	4.4 ± 1.2 de	
Dead/unaccounted	4.7 ± 1.5 de	3.6 ± 1.2 def	4.0 ± 1.2 de	9.0 ± 1.2 bc	
	2 days post-release				$P < 0.01$
T. ramosissima × *T. chinensis*	16.3 ± 1.5 a	13.2 ± 1.2 a	15.8 ± 1.2 a	9.0 ± 1.2 b	
F. johnstonii	0.0 ± 1.5 d	0.0 ± 1.2 d	0.0 ± 1.2 d	0.0 ± 1.2 d	
Cage walls	1.0 ± 1.5 cd	2.0 ± 1.2 cd	1.2 ± 1.2 d	2.0 ± 1.2 cd	
Dead/unaccounted	2.7 ± 1.5 cd	4.8 ± 1.2 c	3.0 ± 1.2 cd	9.0 ± 1.2 b	
	5 days post-release				$P < 0.01$
T. ramosissima × *T. chinensis*	17.0 ± 1.5 a	14.4 ± 1.2 a	16.0 ± 1.2 a	9.6 ± 1.2 b	
F. johnstonii	1.0 ± 1.5 cd	0.0 ± 1.2 d	1.8 ± 1.2 cd	0.0 ± 1.2 d	
Cage walls	0.0 ± 1.5 cd	3.4 ± 1.2 c	0.6 ± 1.2 cd	2.4 ± 1.2 cd	
Dead/unaccounted	2.0 ± 1.5 cd	2.2 ± 1.2 cd	1.6 ± 1.2 cd	8.0 ± 1.2 b	

[a] Tests in screen cages 68 × 53 × 85 (ht) cm, each cage with 20 beetles (10 males, 10 females) and 2 plants (1 *Tamarix* and 1 *Frankenia*); $n = 3$ (Crete) or 5 for each test plant pair and beetle population.
[b] Within each plant pairing and day post-release, individual means (when there was a significant Location × Beetle interaction) followed by the same letter within and between columns are not significantly different. Otherwise, the averages of the means for the four beetle populations within location (averages not listed) followed by the same letter are not significantly different (Fisher's protected least significant difference test, $P > 0.05$).
[c] *P* value for Location × Beetle interaction. In all cases, the main effect of Beetle Population was not significant ($P = 1.0$) whereas Location was highly significant ($P < 0.01$).
[d] Molecular identification; GenBank Accession Nos. AY090385 and AY090386.

F. johnstonii) were randomly arranged in each cage. Average heights (including the 20 cm tall pot) of the plants were 66 cm (saltcedar), 37 cm (*F. jamesii*), and 42 cm (*F. johnstonii*). Pots rested on a 3 cm layer of potting soil that absorbed excess water within the cages to prevent the entrapment and drowning of beetles. Plants were watered with a drip irrigation system (Table 3).

The experimental design for each of the two test plant combinations was a two-way factorial treatment structure in a completely randomized design. Three (Crete only) or five replications were used, with four different *D. elongata* populations and either four possible locations for adults (saltcedar, *Frankenia* plant, cage walls and dead/unaccounted) or three locations for eggs (saltcedar, *Frankenia* plant, and cage walls). Beetles (10 males and 10 females per cage) were released into the center of the cage. Adults were counted 1, 2, or 5 days post-release and their location was recorded. Adults not easily observed for the 1 and 2 day post-release counts were scored as unaccounted to avoid unnecessarily disturbing the beetles. Dead adults were sexed and replaced if found. Eggs were collected, noting their location, on the fifth day post-release to allow for maximum oviposition to occur but just prior to hatching of the oldest eggs. All plants were thoroughly washed with

Table 4
Presence of adult *D. elongata* from three different populations 1, 2, and 5 days post-release on *Tamarix*, *Frankenia*, and willow plants: paired-choice (potential host:non-host) tests in small cages outdoors, Temple, TX, August 2003[a]

Location of adults:	No. adults per location (mean ± SE)[b]			*P* value[c]
	Sfax, Tunisia	Karshi, Uzbekistan	Turpan, China	
	Frankenia jamesii vs. Coyote willow			
	1 day post-release			$P = 0.20$
F. jamesii	0.0 ± 1.4	0.0 ± 1.4	0.0 ± 1.7	c
Coyote willow	0.0 ± 1.4	0.0 ± 1.4	0.0 ± 1.7	c
Cage walls	11.7 ± 1.4	14.7 ± 1.4	13.5 ± 1.7	a
Dead/unaccounted	8.3 ± 1.4	3.0 ± 1.4	6.5 ± 1.7	b
	2 days post-release			$P < 0.01$
F. jamesii	0.0 ± 0.2 d	0.3 ± 1.4 cd	1.0 ± 1.9 bcd	
Coyote willow	0.0 ± 0.2 d	0.0 ± 1.4 d	0.0 ± 1.9 cd	
Cage walls	15.3 ± 0.2 a	4.7 ± 1.4 b	4.0 ± 1.9 bc	
Dead/unaccounted	4.7 ± 0.2 b	12.7 ± 1.4 a	15.0 ± 1.9 a	
	5 days post-release			$P = 0.68$
F. jamesii	3.0 ± 1.4	1.7 ± 1.4	0.5 ± 1.8	b
Coyote willow	0.0 ± 1.4	0.0 ± 1.4	0.0 ± 1.8	b
Cage walls	2.0 ± 1.4	0.3 ± 1.4	1.0 ± 1.8	b
Dead/unaccounted	15.0 ± 1.4	15.7 ± 1.4	18.5 ± 1.8	a
	Frankenia johnstonii vs. Coyote willow			
	1 day post-release			$P = 0.23$
F. johnstonii	0.0 ± 1.4	1.0 ± 1.4	0.0 ± 1.7	c
Coyote willow	0.0 ± 1.4	0.0 ± 1.4	0.0 ± 1.7	c
Cage walls	11.3 ± 1.4	8.6 ± 1.4	14.5 ± 1.7	a
Dead/unaccounted	8.7 ± 1.4	7.7 ± 1.4	5.5 ± 1.7	b
	2 days post-release			$P = 0.26$
F. johnstonii	0.0 ± 2.1	3.0 ± 2.1	2.0 ± 2.6	c
Coyote willow	0.0 ± 2.1	0.0 ± 2.1	0.0 ± 2.6	c
Cage walls	10.3 ± 2.1	3.3 ± 2.1	4.5 ± 2.6	b
Dead/unaccounted	9.7 ± 2.1	11.0 ± 2.1	13.5 ± 2.6	a
	5 days post-release			$P = 0.63$
F. johnstonii	8.3 ± 2.7	5.3 ± 2.7	1.5 ± 3.3	b
Coyote willow	0.0 ± 2.7	0.0 ± 2.7	0.0 ± 3.3	c
Cage walls	0.3 ± 2.7	2.0 ± 2.7	5.0 ± 3.3	bc
Dead/unaccounted	11.3 ± 2.7	10.0 ± 2.7	13.5 ± 3.3	a
	Tamarix ramosissima × *T. chinensis*[d] vs. Coyote willow			
	1 day post-release			$P = 0.33$
T. ramosissima × *T. chinensis*	10.0 ± 2.0	11.3 ± 2.0	17.0 ± 2.5	a
Coyote willow	0.0 ± 2.0	0.0 ± 2.0	0.0 ± 2.5	b
Cage walls	4.7 ± 2.0	3.7 ± 2.0	1.0 ± 2.5	b
Dead/unaccounted	5.3 ± 2.0	2.7 ± 2.0	2.0 ± 2.5	b
	2 days post-release			$P = 0.85$
T. ramosissima × *T. chinensis*	14.0 ± 1.8	10.7 ± 1.8	13.0 ± 2.2	a
Coyote willow	0.0 ± 1.8	0.0 ± 1.8	0.0 ± 2.2	c
Cage walls	2.3 ± 1.8	2.3 ± 1.8	1.0 ± 2.2	c
Dead/unaccounted	3.7 ± 1.8	4.7 ± 1.8	6.0 ± 2.2	b
	5 days post-release			$P = 0.09$
T. ramosissima × *T. chinensis*	17.0 ± 1.1	11.7 ± 1.1	15.5 ± 1.4	a
Coyote willow	0.0 ± 1.1	0.3 ± 1.1	0.0 ± 1.4	c
Cage walls	1.3 ± 1.1	3.3 ± 1.1	1.5 ± 1.4	bc
Dead/unaccounted	1.7 ± 1.1	2.3 ± 1.1	3.0 ± 1.4	b
	Coyote willow vs. Coyote willow			
	1 day post-release			$P = 0.30$
Coyote willow #1	0.3 ± 1.1	0.0 ± 1.1	0.0 ± 1.4	c
Coyote willow #2	0.0 ± 1.1	0.0 ± 1.1	0.0 ± 1.4	c
Cage walls	12.7 ± 1.1	14.7 ± 1.1	15.5 ± 1.4	a
Dead/unaccounted	7.0 ± 1.1	3.3 ± 1.1	4.5 ± 1.4	b

Table 4 (*continued*)

Location of adults:	No. adults per location (mean ± SE)[b]			*P* value[c]
	Sfax, Tunisia	Karshi, Uzbekistan	Turpan, China	
	2 days post-release			$P = 0.15$
Coyote willow #1	0.0 ± 1.7	0.0 ± 1.7	0.0 ± 2.1	b
Coyote willow #2	0.3 ± 1.7	0.0 ± 1.7	0.5 ± 2.1	b
Cage walls	12.7 ± 1.7	6.3 ± 1.7	10.5 ± 2.1	a
Dead/unaccounted	7.0 ± 1.7	11.7 ± 1.7	9.0 ± 2.1	a
	5 days post-release			$P = 0.98$
Coyote willow #1	0.0 ± 0.9	0.0 ± 0.9	0.0 ± 1.0	b
Coyote willow #2	0.0 ± 0.9	0.0 ± 0.9	0.0 ± 1.0	b
Cage walls	1.7 ± 0.9	0.3 ± 0.9	1.5 ± 1.0	b
Dead/unaccounted	18.3 ± 0.9	17.7 ± 0.9	18.5 ± 1.0	a

[a] Tests in screen cages 68 × 53 × 85 (ht) cm, each cage with 12–20 beetles (2–10 males, 10 females) and 2 plants (1 potential host plant and 1 coyote willow [non-host]); $n = 2$ (Turpan) or 3 for each test plant pair and beetle population.
[b] Within each plant pairing and day post-release, individual means (when there was a significant Location × Beetle interaction) followed by the same letter within and between columns are not significantly different. Otherwise, the averages of the means for the three beetle populations within location (averages not listed) followed by the same letter are not significantly different (Fisher's protected least significant difference test, $P > 0.05$).
[c] *P* value for Location × Beetle interaction. In all cases, the main effect of beetle population was not significant ($P > 0.65$) whereas location was highly significant ($P < 0.01$).
[d] Molecular identification; GenBank Accession Nos. AY090385 and AY090386.

water and their locations randomized between replicates. New adults were used for each replicate.

2.4.2. Paired-choice test (potential host:non-host), small cages, outdoors, 2003

This test, conducted in August 2003, was similar to the previous one in design except that adult *D. elongata* presence and oviposition were assessed on *F. jamesii* and *F. johnstonii* in the absence of saltcedar but with a known non-host, coyote willow (*Salix exigua* Nutall) present. Mean minimum and maximum temperatures during the experiment were 22 and 35 °C, respectively. One potted *Frankenia* species (*F. jamesii* or *F. johnstonii*), saltcedar plant (positive control, *T. ramosissima* × *T. chinensis*, Pueblo, CO), or coyote willow (negative control) were paired with a potted coyote willow plant in each cage. The location of each pot within a cage was randomly assigned. Average heights (including the pot) of the plants were 66 cm (saltcedar), 37 cm (*F. jamesii*), 42 cm (*F. johnstonii*), and 65 cm (willow). The experimental design for each test plant combination was a two-way factorial treatment structure in a completely randomized design. Two (Turpan only) or three replicates were used, with three different beetle populations (Tunisia, Uzbekistan or Turpan) and four possible locations for adults (primary test plant, coyote willow, cage walls, and dead/unaccounted) or three locations for eggs (primary test plant, coyote willow, and cage walls). Groups of 10 male and 10 female beetles were released into each cage; a few replicates of Uzbekistan beetles contained fewer males. Adults and eggs were counted as previously described, except that dead adults were not replaced (Table 4).

2.4.3. Multiple-choice tests, small and large cages, outdoors, 2002–2003

Six multiple-choice tests of adult *D. elongata* host preference were conducted from 2002 to 2003. Tests A1–A5 (see Tables 5–7) involved comparisons between *D. elongata* from Crete and the previously released population from Fukang. These tests were conducted from July to August 2002 at a mean minimum and maximum temperature of 23 and 34 °C, respectively. Test A6 (Table 8) consisted of a comparison between *D. elongata* from Tunisia and Uzbekistan and was conducted in September 2003 at a mean minimum and maximum temperature of 18 and 30 °C, respectively. All tests were done in the 3 × 3 × 2 m field cages under a 16:8 h (L:D) photoperiod (natural light supplemented with a halogen floodlight above each cage). This was done primarily to prevent the induction of diapause in the Fukang population. The experimental design for each test was a two-way factorial treatment structure in a completely randomized design, with two *D. elongata* populations and seven to nine possible locations for adults or eggs (5–7 different test plants, cage walls, and ground). We used 4–24 replicates depending on the particular test. Tests A1, A2, A5, and A6 included various species or hybrids of *Tamarix* and the *Frankenia* species *F. salina*, *F. johnstonii* and (except for A6) *F. jamesii*. Test A3 included one *Tamarix* hybrid, the three *Frankenia* species and two species of non-hosts. This test used *Tamarix* plants that were similar in height to the *Frankenia* plants. Test A4 was similar to test A3 except that the *Tamarix* plant was replaced by a third non-host plant to assess the beetles' response in the absence of the target weed (see Tables 5–8 for test plant names).

For tests A1–A4 and A6, which were conducted in the large field cages, each cage was subdivided into four quadrats, each quadrat containing one each of the test plants in pots. The plants were randomly arranged in each quadrat and sunk into the ground to the top of each pot. For each beetle population, 79–125 unsexed adults were released in

Table 5
Presence of adults and oviposition by *D. elongata* from two populations on *Tamarix* and *Frankenia* plants: multiple-choice tests in large outdoor cages, Temple, TX, July-August 2002[a]

Location (test plant, cage walls, and ground)[b]	Percent adults observed or eggs laid (mean ± SD) per location (total per test)									
	Test A1 ($n = 8$)				Test A2 ($n = 8$)					
	Adults		Eggs		Adults			Eggs		
	Crete, Greece	Fukang, China	Crete, Greece	Fukang, China	Crete, Greece	Fukang, China		Crete, Greece	Fukang, China	
Tamarix ramosissima Pueblo, CO	22.1 ± 3.8 bc (184)	28.4 ± 5.6 a (211)	28.2 ± 15.7 bcd (702)	42.4 ± 12.9 a (595)	16.7 ± 5.5 (142)	17.2 ± 6.5 (150)	b	22.1 ± 5.2 (530)	31.0 ± 17.9 (473)	a
T. chinensis Seymour, TX	29.7 ± 8.4 a (253)	24.2 ± 6.6 ab (195)	32.8 ± 9.8 ab (922)	21.4 ± 9.9 cd (281)	—	—	—	—	—	—
T. chinensis and/or *T. ramosissima* X*T. chinensis* Artesia, NM	—	—	—	—	17.3 ± 3.9 (146)	16.4 ± 4.9 (117)	b	17.9 ± 7.9 (388)	17.3 ± 13.1 (282)	b
T. ramosissima and/or *T. ramosissima* × *T. chinensis* Bishop, CA	—	—	—	—	32.6 ± 9.9 (306)	27.3 ± 4.8 (202)	a	33.9 ± 8.1 (870)	24.5 ± 12.3 (377)	a
T. canariensis/*T. gallica* Texas City, TX	26.5 ± 6.6 ab (255)	17.7 ± 5.2 cd (122)	30.7 ± 10.9 abc (1,258)	22.0 ± 12.8 d (399)	—	—	—	—	—	—
T. parviflora Las Cruces, NM	—	—	—	—	17.0 ± 2.3 (147)	15.0 ± 4.6 (118)	b	20.7 ± 11.3 (697)	21.0 ± 11.5 (392)	ab
T. aphylla (athel) Phoenix, AZ	14.0 ± 6.6 de (106)	10.3 ± 3.2 e (98)	7.1 ± 6.4 ef (269)	9.0 ± 4.6 e (202)	—	—	—	—	—	—
Frankenia salina Bishop (Poleta Canyon), CA	0.4 ± 0.8 gh (3)	0.2 ± 0.6 gh (1)	0.0 ± 0.0 g (0)	0.0 ± 0.0 g (0)	0.4 ± 1.3 (1)	0.2 ± 0.4 (2)	c	2.3 ± 4.4 (28)	0.5 ± 1.5 (5)	cd
F. johnstonii Laredo, TX	0.3 ± 0.8 gh (2)	0.3 ± 0.9 gh (2)	0.0 ± 0.0 g (0)	0.0 ± 0.0 g (0)	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c	0.0 ± 0.0 (0)	1.1 ± 3.0 (10)	d
F. jamesii Pueblo, CO	0.0 ± 0.0 h (0)	0.0 ± 0.0 h (0)	0.0 ± 0.0 g (0)	0.0 ± 0.0 g (0)	0.1 ± 0.4 (2)	0.2 ± 0.6 (1)	c	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	d
Cage walls	5.3 ± 4.1 f (45)	18.7 ± 8.1 cd (194)	1.2 ± 3.3 g (13)	5.1 ± 5.7 f (159)	15.4 ± 12.6 (125)	23.2 ± 8.3 (203)	b	2.9 ± 5.3 (113)	4.7 ± 5.6 (111)	c
Ground/weeds	1.8 ± 3.9 gh (7)	0.2 ± 0.4 gh (1)	0.0 ± 0.0 g (0)	0.0 ± 0.0 g (0)	0.4 ± 0.7 (4)	0.4 ± 0.9 (4)	c	0.1 ± 0.4 (16)	0.0 ± 0.0 (0)	d

Factor	*P* value	*P* value	*P* value	*P* value
Location of adults/eggs	0.85	0.33	<0.01	<0.01
Beetle population	<0.01	<0.01	0.53	0.85
Location × Beetle	<0.01	<0.01	0.24	0.51

[a] Within each test and life stage, individual means (when there was a significant Location × Beetle interaction) followed by the same letter within and between the Crete and Fukang columns are not significantly different. Otherwise, the averages of the means for the two beetle populations within location (averages not listed) followed by the same letter are not significantly different (two-way Kruskal–Wallis Test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$). Each replicate (representing a 24 h period) is the average of four quadrats in a cage.

[b] Molecular identifications of *Tamarix*. Accession numbers are given in Table 1.

Table 6
Presence of adults and oviposition by *D. elongata* from two populations on *Tamarix*, *Frankenia* and non-host plants: multiple-choice tests in large outdoor cages, Temple, TX, July–August 2002[a]

Location (test plant, cage walls, and ground)	Percent adults observed or eggs laid (mean ± SD) per location (total per test)											
	Test A3 ($n=4$)						Test A4 ($n=8$ Crete or 5 Fukang)					
	Adults			Eggs			Adults			Eggs		
	Crete, Greece	Fukang, China		Crete, Greece	Fukang, China		Crete, Greece	Fukang, China		Crete, Greece	Fukang, China	
[b]*T. chinensis* × *T. canariensis*/*T. gallica* Pecos River/I-10, TX	16.9 ± 3.0 (58)	22.3 ± 22.6 (52)	b	20.5 ± 15.9 (139)	31.6 ± 31.6 (132)	b	—	—	—	—	—	—
Frankenia salina Bishop (Poleta Canyon), CA	0.6 ± 1.3 (3)	0.3 ± 0.6 (1)	c	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c	1.2 ± 2.2 (9)	0.5 ± 0.8 (2)	bc	3.5 ± 6.6 (85)	5.6 ± 7.9 (41)	b
F. johnstonii Laredo, TX	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	d	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c	0.1 ± 0.3 (1)	0.0 ± 0.0 (0)	de	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c
F. jamesii Pueblo, CO	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	d	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	e	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c
Limonium limbatum	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	d	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	e	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c
Plumbago capensis	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	d	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c	0.7 ± 0.7 (6)	0.0 ± 0.0 (0)	bcd	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c
Salix gooddingii	—	—	—	—	—	—	0.9 ± 2.3 (5)	0.0 ± 0.0 (0)	cde	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c
Cage walls	81.5 ± 2.1 (267)	77.4 ± 22.4 (242)	a	79.5 ± 15.9 (449)	68.4 ± 31.6 (155)	a	94.6 ± 3.6 (670)	97.8 ± 3.5 (430)	a	96.5 ± 6.6 (1,114)	94.4 ± 7.9 (391)	a
Ground/weeds	0.9 ± 1.1 (3)	0.0 ± 0.0 (0)	c	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c	2.5 ± 3.0 (23)	1.7 ± 3.7 (2)	b	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	c
Factor	*P* value			*P* value			*P* value			*P* value		
Location of adults/eggs	<0.01			<0.01			<0.01			<0.01		
Beetle population	0.27			1.00			0.01			0.65		
Location × Beetle	0.38			0.99			0.10			0.96		

[a] Within each test and life stage, the averages of the means for the two beetle populations within location (averages not listed) followed by the same letter are not significantly different (two-way Kruskal–Wallis test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P>0.05$). Each replicate (representing a 24h period) is the average of four quadrats in a cage.
[b] Molecular identification; GenBank Accession Nos. AY090386 and AY090437.

the center of a cage. After approximately 24 h, or 2–4 days for test A6 only, the number of adults and eggs on each test plant and the cage walls and ground were recorded for each quadrat. Eggs were removed and adults were aspirated off the plants. Adults, previously used and new, for each population were then released back into the same cage (tests A3 and A6) or were rotated between different tests that were run simultaneously (tests A1, A2, and A4). The data analyzed were the average of the four quadrats for each observation date.

Test A5 (small cage test) used three aluminum screen cages, 68 × 53 × 85 cm, placed in a large field cage. Five potted test plant species were randomly arranged in each small cage. Pots were set on a 3 cm layer of dried grass overlaying a 3 cm layer of soil. Between 36 and 40 adults were placed in the center of each small cage for approximately 24 h, after which all adults and eggs were removed and their locations recorded. Adults, previously used in other adult tests, were released back into the small cages for additional replicates. For all tests, due to the variable number of adults available between beetle populations and replicates and the unknown proportion of females, adult colonization and oviposition data were converted to the percent of adults or eggs, respectively, found at each location for a given replicate. The average height of plants above the soil line for tests A1–A5 was 80 cm for *Tamarix* (20 cm for test A3), 20 cm for *Frankenia,* 13 cm for *Limonium*, 60 cm for *Plumbago*, and 130 cm for *Salix*. In test A6, the average height of plants was 76 cm for *Tamarix* and 27 cm for *Frankenia*.

2.5. Statistical analyses

Data on percentage larval/pupal survival and percentage of adults or eggs per location (each test plant species, cage wall, and ground) did not generally conform to the assumptions of parametric tests and were analyzed using a protected Kruskal–Wallis test performed on the ranks of the percentage data (PROC GLM, SAS Institute, 1999). Data on the number of adults or eggs per location (each test plant, cage walls, and dead/unaccounted) were subjected to analysis of variance (PROC MIXED, SAS Institute, 1999). Means (numbers or ranks) were separated using Fisher's protected least significant difference test (LSMEANS, SAS Institute, 1999).

3. Results

3.1. Larval no-choice tests

3.1.1. Vials, laboratory, 2002 (Table 1)

Percentage survival (neonate to adult) of larval *D. elongata* from Crete restricted to a single test plant was generally similar among the various *Tamarix* species, accessions and hybrids, including between the deciduous saltcedars (except for *T. parviflora*) and the evergreen athel (*T. aphylla*). Survival was higher on *Tamarix* plants than on all other test plants,

Table 7
Test A5, presence of adults and oviposition by *D. elongata* from two populations on *Tamarix* and *Frankenia* plants: multiple-choice test in small outdoor cages, Temple, TX, July–August 2002[a]

Location (test plant, cage walls, and ground)	Percent (mean ± SD) observed per location (total per test)			
	Adults		Eggs	
	Crete, Greece ($n = 24$)	Fukang, China ($n = 18$)	Crete, Greece ($n = 24$)	Fukang, China ($n = 18$)
[b]*Tamarix ramosissima* Pueblo, CO	41.3 ± 14.7 a (381)	41.2 ± 17.3 a (274)	33.9 ± 19.0 a (1,392)	47.7 ± 26.4 a (674)
T. aphylla (athel) Phoenix, AZ	39.2 ± 14.9 a (369)	34.4 ± 15.9 a (226)	44.8 ± 20.8 a (1,921)	40.1 ± 27.6 a (585)
Frankenia salina Bishop (Poleta Canyon), CA	2.5 ± 2.8 d (23)	0.9 ± 1.8 ef (6)	4.3 ± 7.3 c (212)	0.7 ± 2.9 d (15)
F. johnstonii Laredo, TX	0.4 ± 1.3 ef (4)	0.0 ± 0.0 f (0)	0.4 ± 2.1 d (24)	0.0 ± 0.0 d (0)
F. jamesii Pueblo, CO	0.0 ± 0.0 f (0)	0.4 ± 1.3 ef (3)	0.0 ± 0.0 d (0)	1.2 ± 4.9 d (24)
Cage walls	15.3 ± 8.6 c (140)	21.6 ± 10.2 b (142)	16.3 ± 17.5 b (613)	8.9 ± 10.9 c (145)
Ground/weeds	1.2 ± 2.1 e (11)	1.5 ± 3.7 ef (10)	0.3 ± 1.3 d (18)	1.4 ± 6.1 d (6)
Factor	*P* value		*P* value	
Location of adults/eggs	<0.01		<0.01	
Beetle population	0.23		0.08	
Location*Beetle	0.02		0.04	

[a] For each life stage, individual means followed by the same letter within and between the Crete and Fukang columns are not significantly different (Two-way Kruskal–Wallis Test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$). Each replicate (representing a 24 h period) is one cage.
[b] Molecular identification; GenBank Accession No. AY090385.

except between Crete larvae fed *T. canariensis*/*T. gallica* and *F. salina*. Survival also was higher on *F. salina* compared to *F. jamesii*, *F. johnstonii* and the remaining test plants. All Crete larvae provided plants other than *Tamarix* or *Frankenia* (order Tamaricales) died as 1st instars within 3 days.

No differences were found in percentage survival of *D. elongata* from Fukang among the various *Tamarix* test plants. Larval/pupal survival on *Tamarix* was higher than on the three *Frankenia* species, except for *T. parviflora*. Only one larva of 10 survived to the adult stage on *F. salina* and all Fukang larvae died as 1st instars on *F. jamesii* and *F. johnstonii*.

We performed a separate two-way Kruskal–Wallis Test on the ranked percentage data that included beetle population and Tamaricales plants (*Tamarix* and *Frankenia* species only) as factors. *D. elongata* from Crete had a higher average survival rate than beetles from Fukang (62 and 49%, respectively; $P < 0.032$). The pattern of survival among test plants was similar between the two beetle populations, with greater survival on *Tamarix* plants than on *Frankenia* plants ($P < 0.020$, Fisher's Protected LSD test).

3.1.2. Agricultural plants, vials, laboratory, 2003

All larvae of *D. elongata*, regardless of beetle population, died within 4 days as 1st instars when fed excised leaves from the various species of agricultural plants. Percentage survival of larvae fed *T. ramosissima* was moderate to high for all beetle populations in comparison [62.5 ± 14.4% (Crete), 93.8 ± 12.5% (Tunisia), 68.8 ± 12.5% (Uzbekistan), and 87.5 ± 25.0% (Turpan)].

3.1.3. Non-Tamaricales plants, vials, laboratory, 2003

No larvae survived more than 3 days, remaining as 1st instars, when provided leaves from different taxonomically related (non-Tamaricales) plants of saltcedar (i.e., not including the *Frankenia* species) or unrelated habitat associates. As in the previous test, percentage survival to the adult stage was moderate to high for all populations of larvae fed *T. ramosissima* [93.8 ± 12.5% (Tunisia), 68.8 ± 12.5% (Uzbekistan), and 45.8 ± 36.3% (Turpan)]. Although feeding damage was not examined in this and the previous tests, larvae of *D. elongata* provided non-host plants generally were observed to wander continuously in the vials until death. In contrast, larvae provided saltcedar settled on the leaves and began feeding within an hour of the start of the experiment.

3.1.4. Tamaricales, sleeve bags, outdoors, 2003 (Table 2)

The greatest differences in survival were observed with *D. elongata* from Tunisia. Larval/pupal survival for Tunisia beetles was generally similar among the various *Tamarix* test plants. Survival rates on *Frankenia* plants were much less than those on the different saltcedars but were similar to that on athel. In contrast, *D. elongata* from Uzbekistan only displayed some differences in survival rates among the various *Tamarix* and *Frankenia* plants. In particular, percentage survival on athel was greater than on two of the saltcedar accessions and the three *Frankenia* species. No differences were found in percentage survival rates, which were moderate to high, for Turpan larvae fed various *Tamarix* and *Frankenia* plants.

3.2. Adult tests

3.2.1. Paired-choice test (Tamarix:Frankenia), small cages, outdoors, 2003

The presence of adult *D. elongata* on saltcedar (*T. ramosissima* × *T. chinensis*) and *Frankenia* plants was generally not different among the beetles from Crete, Tunisia, Uzbekistan and Turpan for each sampling date. An unexplained higher mortality of Turpan beetles in some replicates involving *F. johnstonii* led to generally lower

Table 8
Test A6, presence of adults and oviposition by *D. elongata* from two populations on *Tamarix* and *Frankenia* plants: multiple-choice test in large outdoor cages, Temple, TX, September 2003[a]

	Percent (mean ± SD) observed per location (total per test) ($n = 5$)				
	Adults			Eggs	
Location (test plant, cage walls, and ground)[b]	Sfax, Tunisia	Karshi, Uzbekistan		Sfax, Tunisia	Karshi, Uzbekistan
Tamarix parviflora Las Cruces, NM	24.2 ± 10.4 (107)	24.4 ± 8.2 (87)	a	33.2 ± 15.0 a (1254)	17.3 ± 10.1 b (961)
T. chinensis × *T. canariensis*/*T. gallica* Big Spring, TX (AY090386, AY090437)	23.3 ± 5.2 (106)	17.3 ± 7.9 (79)	ab	25.9 ± 5.3 ab (1195)	22.0 ± 9.0 ab (1344)
T. ramosissima Salt Creek, CA (AY090385)	17.7 ± 6.4 (81)	21.5 ± 5.6 (93)	ab	17.5 ± 8.8 ab (713)	27.1 ± 11.3 ab (1331)
T. aphylla (athel) Encino, TX	14.8 ± 8.9 (71)	15.8 ± 1.1 (64)	b	6.8 ± 7.5 cd (395)	19.4 ± 10.6 ab (1023)
T. canariensis/*T. gallica* Galveston, TX (AY090398, AY090437)	10.2 ± 2.2 (55)	9.7 ± 3.5 (122)	c	16.4 ± 5.8 b (667)	6.7 ± 2.8 c (122)
Frankenia salina Owens Valley, CA	0.0 ± 0.0 (0)	0.9 ± 0.9 (4)	d	0.0 ± 0.0 f (0)	1.4 ± 1.9 def (107)
F. johnstonii Laredo, TX	0.0 ± 0.0 (0)	0.4 ± 0.6 (2)	d	0.0 ± 0.0 f (0)	0.0 ± 0.0 f (0)
Cage walls	7.9 ± 3.4 (35)	9.7 ± 4.1 (42)	c	0.2 ± 0.4 ef (10)	4.3 ± 5.5 cde (178)
Ground	1.9 ± 2.3 (7)	0.4 ± 0.9 (1)	d	0.0 ± 6.6 f (0)	1.7 ± 3.8 ef (71)
Factor	*P* value			*P* value	
Location of adults/eggs	<0.01			<0.01	
Beetle population	0.46			0.20	
Location × Beetle	0.28			<0.01	

[a] For each life stage, individual means (eggs, a significant Location × Beetle interaction) followed by the same letter within and between the Tunisia and Uzbekistan columns are not significantly different. Otherwise, the averages of the means for the two beetle populations within location (adults, averages not listed) followed by the same letter are not significantly different (two-way Kruskal–Wallis test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$). Each replicate (representing a 2–4 day period) is the average of four quadrats in a cage.
[b] Molecular identifications of *Tamarix* with GenBank accession numbers, if available. X denotes a hybrid.

numbers of Turpan adults on saltcedar plants compared to the other beetle populations. The number of adult beetles, when given a choice between saltcedar and one of two *Frankenia* species, was higher on the saltcedar plant 1, 2, and 5 days post-release than on the *Frankenia* plants or cage walls, with only one exception on the first day (Turpan beetles on cage walls with *F. johnstonii* present) (Table 3). No to very few adults were counted on the *Frankenia* plants at any time, which was not different or less than the number of adults on the cage walls (Table 3).

Oviposition by all populations of *D. elongata* was much greater on the saltcedar plants, averaging 355–545 eggs per plant, than on either *Frankenia* species, on which zero to a maximum average of five eggs were laid per plant (Fig. 1). Also, oviposition on the *Frankenia* plants was not different than on the surrounding cage walls (Fig. 1). A three-way factorial analysis, with beetle population, *Frankenia* species and location of eggs as factors, revealed a significant *Frankenia* × location interaction ($P < 0.009$). This was due to greater oviposition by *D. elongata* on saltcedar plants paired with *F. jamesii* than on saltcedar paired with *F. johnstonii*. Therefore, all beetle populations responded similarly in this test regarding the acceptability of the two native *Frankenia* plants for oviposition.

3.2.2. Paired-choice test (potential host:non-host), small cages, outdoors, 2003

The presence of adult *D. elongata* on the different plants within each of the four test plant combinations was similar (one exception) among the three beetle populations we tested. The number of adult beetles was greatest on the saltcedar plant (*T. ramosissima* × *T. chinensis*, positive control cages) than elsewhere in the cage beginning one day after release of the adults and continuing until the end of the experiment (Table 4). Survival of adults was high in the presence of saltcedar (85–92%). In contrast, in the remaining cages which contained only *Frankenia* (potential host) and willow (non-host) plants, more adults were found on the cage walls 24 h following release than on the *Frankenia* or willow plants (Table 4). By the 2nd day post-release, the numbers shifted toward more dead or unaccounted adults with continued low numbers or no adults on the plants. After 5 days, most adult beetles were dead or were not recovered and presumably had died (57–93% of adults in *Frankenia* cages; 92–98% in willow only cage). The number of adults on the *Frankenia* plants was less than the number that had died but similar to the number still alive on the cage walls (Table 4). Some feeding by adult *D. elongata* was noted on the *Frankenia* plants.

Oviposition by all populations of *D. elongata*, in the absence of saltcedar, was low on either *Frankenia* species (averaging 0–50 eggs) and not different than that on the non-host coyote willow or on the cage walls (Fig. 2). In contrast, on the saltcedar plants (positive control), the adults laid large numbers of eggs, averaging 355–700 eggs per plant (Fig. 2). A separate comparison of oviposition among saltcedar, the two *Frankenia* species and coyote willow only for each beetle type showed that, regardless of the original source of beetles, saltcedar received more eggs than all other test plants, whereas the number of eggs laid on the *Frankenia* or willow plants was not different (Fig. 2). Additionally, oviposition on the two *Frankenia* species did not differ among beetle populations ($P > 0.51$, two-way factorial analysis with beetle population and egg location as factors).

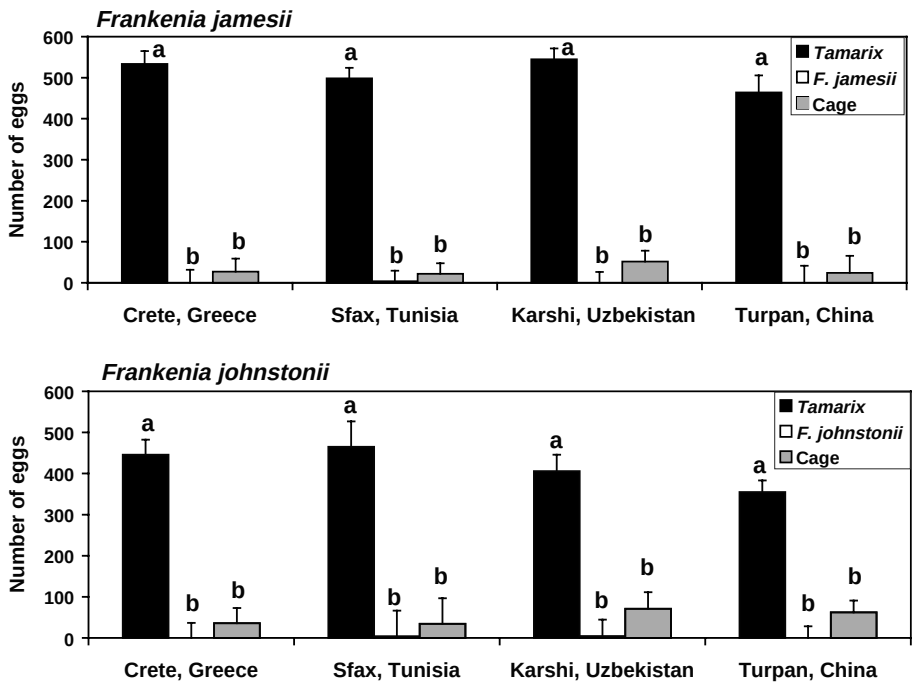

Fig. 1. Oviposition by *D. elongata* (mean + SE number of eggs) from four different populations after 5 days on *T. ramosissima* × *T. chinensis*, a *Frankenia* species, and cage walls: paired-choice (*Tamarix*:*Frankenia*) tests, Temple, TX, July 2003. *Frankenia jamesii* (top) and *F. johnstonii* (bottom). For each *Frankenia* species and beetle population, bars denoted by the same letter are not significantly different (Fisher's protected LSD, $P > 0.05$).

3.2.3. Multiple-choice tests, small and large cages, outdoors, 2002-2003

Tests A1 and A2 compared adult preference by Crete and Fukang *D. elongata* for different species or hybrids of saltcedar, athel and three species of *Frankenia* in the large outdoor cages. For test A1, percentage adult presence for both beetle types was greater on the three saltcedar accessions than on athel (Table 5). In turn, adult presence on all *Tamarix* test plants was greater than on the three *Frankenia* species, on which only a few adult beetles were found over the duration of the test (Table 5). Furthermore, an equal or greater percentage of adults was found on the cage walls and surrounding ground inside the cage compared to the *Frankenia* plants (Table 5). Oviposition among test plants by both beetle populations showed a similar pattern, with a greater percentage of *D. elongata* eggs being laid on the three saltcedar species than on athel. Oviposition on athel was approximately 27% of that on the saltcedar species used in test A1. No eggs were found on the *Frankenia* plants (Table 5). The two beetle types differed in their response to certain saltcedar species. Both the percent of adults and eggs were higher on *T. canariensis*/*T. gallica* and lower on *T. ramosissima* for beetles from Crete compared to beetles from Fukang. Additionally, percent oviposition was greater on *T. chinensis* by Crete beetles compared to Fukang beetles (Table 5).

In Test A2, the two beetle populations did not differ in their responses to the test plants. Similar to the results of test A1, the percentage of adults and eggs present on the four *Tamarix* test plants were higher than on all three *Frankenia* species (Table 5). Also, the very low percentage of adults and eggs on *Frankenia* plants were equal to or less than that on the cage walls (Table 5). Among *Tamarix* accessions, adult presence was highest on *T. ramosissima*/*T. ramosissima* × *T. chinensis,* although this was not necessarily accompanied by greater oviposition (Table 5).

In Test A3, as in the previous test, we found no difference in the response of the Crete and Fukang populations. Most of the adult beetles and eggs were found on the cage walls (over 68%, Table 6). The percentage of adults on the sole *Tamarix* test plant, which was similar in height to the *Frankenia* plants, was much greater than adult colonization on *F. salina*, which was the only other test plant on which adults were found (Table 6). No eggs were found on any of the *Frankenia* species or the two non-host plants (Table 6).

Test A4 was the companion study to test A3 in which the saltcedar plant was replaced with a non-host plant. In the absence of the target weed, adults and eggs for both Crete and Fukang beetles were almost all found on the cage walls (over 94%, Table 6). The average percentage of adults on *F. salina* was equal to that on the non-hosts *Plumbago* and *Salix* and greater than on the other two *Frankenia* species and *Limonium* (Table 6). Limited oviposition occurred on *F. salina* but not on any other plants (Table 6).

In test A5, which was conducted in small cages, the percentage presence of adults and eggs were greater on the two *Tamarix* species (a saltcedar and athel) than on the cage walls (Table 7). In turn, the percent of adults and eggs found on the cage walls was greater than on the three *Frankenia* species (Table 7). The two beetle populations differed in that a higher percent of Crete adults and eggs were found on *F. salina* compared to beetles from Fukang (Table 7).

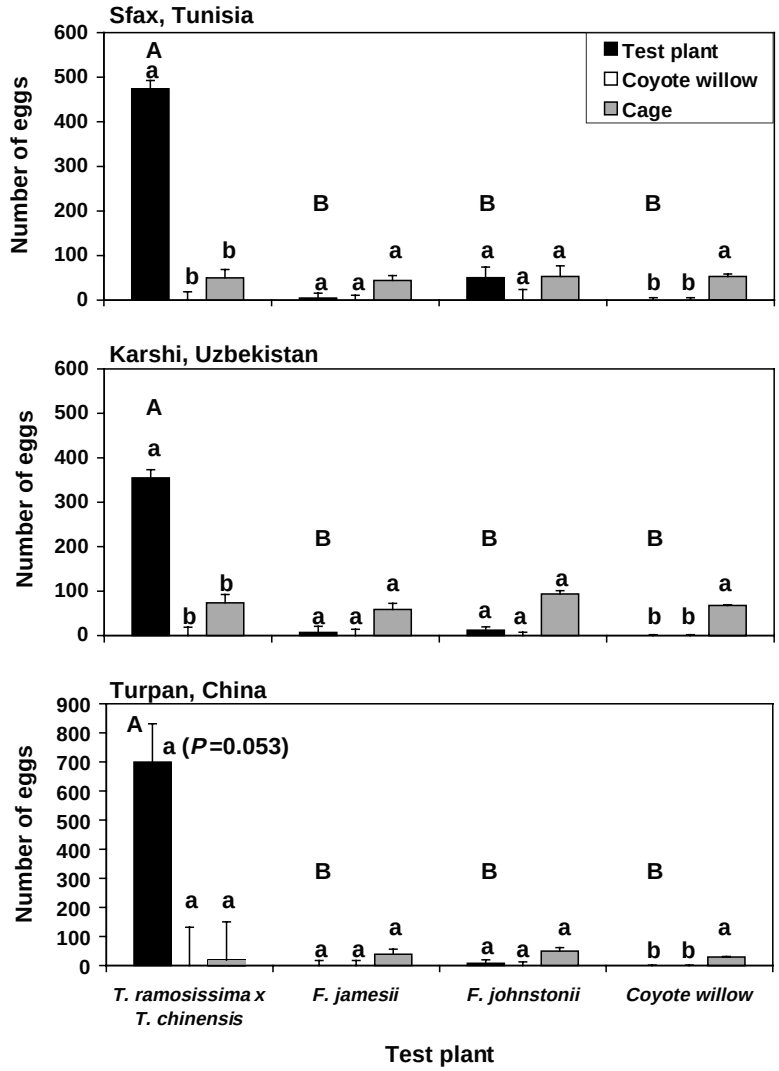

Fig. 2. Oviposition by *D. elongata* (mean + SE number of eggs) from three different populations after 5 days on *T. ramosissima* × *T. chinensis*, *Frankenia* plants, coyote willow, and cage walls: paired-choice (potential host:non-host) tests, Temple, TX, August 2003. *D. elongata* from Sfax, Tunisia (top), Karshi, Uzbekistan (middle), and Turpan, China (bottom). Uppercase letters—for each beetle population, primary test plant bars (black) denoted by the same letter are not significantly different. Lowercase letters—for each beetle population and test plant pair, bars denoted by the same letter are not significantly different (Fisher's protected LSD, $P > 0.05$).

Test A6 was the only multiple-choice test we conducted involving *D. elongata* from Tunisia and Uzbekistan. Adult colonization of plants was not different between the two beetle populations. The percent of adults present on the *Frankenia* plants was lower than on all *Tamarix* test plants as well as the cage walls (Table 8). Among the *Tamarix*, more adults were found on *T. parviflora* than on athel or *T. canariensis*/*T. gallica* (Table 8). Within each beetle population, percent oviposition on *Frankenia* plants, which included no eggs, was less than on *Tamarix* plants and similar to or less than oviposition on the cage walls (Table 8). Specifically, oviposition only occurred on *F. salina* by beetles from Uzbekistan. The two populations differed in their ovipositional response to the various *Tamarix* accessions. Beetles from Tunisia laid a lower percentage of eggs on athel than the saltcedars (Table 8). In contrast, egg-laying by Uzbekistan beetles was similar between the various saltcedars and athel with the exception of lower oviposition on *T. canariensis*/*T. gallica* (Table 8). Furthermore, Tunisia *D. elongata* laid a higher percentage of eggs on *T. parviflora* and *T. canariensis*/*T. gallica*, but a lower percentage of eggs on athel, compared to Uzbekistan beetles (Table 8).

4. Discussion

The host ranges of the four southern populations of the leaf beetle *D. elongata* that we tested (from Tunisia, Crete, Uzbekistan, and Turpan, China) are very similar to each other and to the previously released populations from Fukang, China and Chilik, Kazakhstan (DeLoach et al., 2003; Lewis et al., 2003a). This includes the host specificity displayed by both the larval and adult stages.

4.1. Larval host range

The physiological, or fundamental, host range for *D. elongata* larvae in North America includes plants in the genera *Tamarix* and *Frankenia*, both in the order Tamaricales but placed in the families Tamaricaceae and Frankeniaceae, respectively. These are the only two genera of Tamaricales found in North America and only *Frankenia* is native; another Eastern Hemisphere genus (*Myricaria*) also can support some larval development (Medvedev, 1982; DeLoach et al., 2003). The results of our larval no-choice tests reported here are similar to those of DeLoach et al. (2003) and Lewis et al. (2003a) for *D. elongata* from Fukang, China and Chilik, Kazakhstan, released into the open field in 2001 and now established in Nevada, Utah, Colorado and Wyoming (DeLoach et al., 2004).

All *Tamarix* species and hybrids that are invasive in the United States and which we assessed were generally suitable for complete larval development among the populations of *D. elongata* we tested, although a few differences were sometimes evident. For example, athel, the large evergreen *Tamarix* species that is not currently being targeted for control, was equally suitable, as measured by larval/pupal survival, compared to the deciduous saltcedars for *D. elongata* from Fukang or Turpan (China) and Crete, was better than a few saltcedar accessions for beetles from Uzbekistan, and was a relatively but not significantly poorer host for *D. elongata* from Tunisia. However, the variability of results precludes any robust ranking of *Tamarix* species and hybrids among the various beetle populations, if indeed any ranking is possible for the larvae. Nevertheless, Lewis et al. (2003b) reported that larval feeding on different *Tamarix* plants significantly affected adult fecundity of *D. elongata* females, which might influence the level of suppression of different saltcedars present in the North American infestation.

Larval *D. elongata* can develop successfully to varying degrees on the three *Frankenia* species included in our tests, and probably can on the remaining three species native to North America that we did not test (Whalen, 1980, 1987). Survival was often poor among beetle populations (0–18%), including no larval development, but it could be quite high on all *Frankenia* species, up to 76%, as we observed for *D. elongata* from Turpan. Similarly variable survival rates (0–60%) were reported by Lewis et al. (2003a) for a single population (*D. elongata* from Fukang). Among *Frankenia* species, we found a trend for greater survival on *F. salina* compared to *F. johnstonii* and especially to *F. jamesii*. Differences in host plant quality among tests, and perhaps among *Frankenia* species, may contribute to variability in survival rates (Lewis et al., 2003a). The use of cut leaves or whole plants did not seem to contribute to this variability. This raises the question as to how the potential quality of *Frankenia* plants grown and maintained in the laboratory or greenhouse compares to plants growing in the field. If field grown plants typically are of variable quality, then our results are indicative of the range of possible outcomes of larval feeding, should oviposition occur on *Frankenia* (see below). If field grown plants are consistently of lower quality, as indicated by poorer survival, then the risk posed to *Frankenia* by *D. elongata* larvae would be decreased although not necessarily eliminated. Furthermore, the overall degree to which *Frankenia* are adequate larval hosts remains unclear, as we did not examine the effects of a larval diet of *Frankenia* on adult size, longevity, fecundity or host plant selection for *D. elongata* from Tunisia, Crete, Uzbekistan, and Turpan. However, Lewis et al. (2003a) did report that *D. elongata* from Fukang, when reared as larvae on three species of *Frankenia* for one generation, showed no increased selection of *Frankenia* plants as adults compared to larvae reared on *T. ramosissima*. In addition, larvae initially reared on *T. ramosissima* and then switched to *F. salina* to complete their development had greatly reduced oviposition on saltcedar (Lewis et al., 2003a).

No larval development or survival was recorded on the 28 other species of plants tested. Therefore, we regard plants outside the order Tamaricales as non-hosts for all populations of *D. elongata* and did not test adult beetles against these plants.

4.2. Adult host range

Adult beetles for all populations of *D. elongata* displayed a high degree of discrimination between *Tamarix* and *Frankenia*. Among all the adult tests, few to no adults or eggs were counted on the three *Frankenia* species, in contrast to the saltcedar accessions, even after several days in the absence of saltcedar and the confines of a small cage. Hence, the lack of the target weed for an extended period did not cause the beetles to redirect their egg-laying onto *Frankenia* (Withers et al., 2000; Lewis et al., 2003a). Furthermore, in all our tests conducted in both small and large cages, the beetles laid equal or greater numbers/percentages of eggs on the cage walls than on the *Frankenia* plants. It is not uncommon for female beetles to lay some eggs even in empty vials when artificially confined in the vial for a period of time. Thus, *Frankenia* species do not appear to be a preferred oviposition substrate for *D. elongata* populations collected across a wide range of its Old World distribution (Lewis et al., 2003a). Kovalev (1995), based on an extensive review of faunal lists for Tamaricaceae, noted only two insects in the genera *Tamaricella* Zachv. and *Ornativalva* Gozm. that were recorded from both *Tamarix* and *Frankenia*. Alan Kirk and Rouhollah Sobhian (ARS European Biological Control Laboratory, Montferrier-sur-Lez, France) found *D. elongata* beetles abundant on *Tamarix* spp. but found none on adjacent *Frankenia* plants in 2000 near Sfax, Tunisia. Additional field surveys have yet to record *D. elongata* from *Frankenia* species in the Old World (see DeLoach et al., 2003).

Frankenia plants also appear to be poor hosts for the maintenance of *D. elongata* adults. In the paired-choice adult tests that lasted 5 days, all adult beetles had been previously maintained on saltcedar and were well fed prior to

the start of the test. Nevertheless, most of the adults died when only *Frankenia* and willow plants were available, whereas nearly all adults survived when saltcedar plants were present. Similarly, Lewis et al. (2003a) reported that beetles from Fukang had reduced adult longevity, did not mate and laid no eggs when adults were maintained solely on any of three *Frankenia* species compared to saltcedar, regardless of whether the larvae had been reared on *Frankenia* or saltcedar.

We did observe a generally non-significant trend for more eggs to be laid on *F. salina* than the other *Frankenia* species, similar to the results for larval survival. How this might translate to an increased risk of damage to *F. salina* in the open field is being investigated more closely at the ARS laboratory in Albany, CA. *Frankenia salina* is common in saline, marshy areas and alkali sinks in the desert in California and Baja California (Whalen, 1980; Whalen, 1987). In a separate study, Dudley and Kazmer (2005) reported only minor feeding and no oviposition on transplanted *F. salina* in an open field test with high populations of the Fukang beetle.

We cannot state conclusively that certain saltcedar species or hybrids are more or less preferred for oviposition for the different populations of *D. elongata*, as the primary effort was to determine the risk to *Frankenia*. *D. elongata* appears to be sufficiently oligophagous to adopt all types of saltcedar although different control outcomes cannot be predicted at this time. Adult beetles from three populations (Tunisia, Crete, and Fukang) did show a preference for the various saltcedars over athel in the large cage, multiple-choice tests, laying approximately one-third the amount of eggs on athel compared to saltcedar. These results are generally consistent with those reported for the Fukang/Chilik beetles (Lewis et al., 2003a). In contrast, the beetles from Uzbekistan displayed no such preference between athel and saltcedar in one test. Also, athel and *T. ramosissima* received similar numbers of eggs in the small cage tests involving *D. elongata* from Crete and Fukang. Furthermore, any discrimination between saltcedar and athel may not necessarily occur in a no-choice test, which was not conducted. Therefore, the potential risk to athel is being further assessed (L.R. Milbrath and C.J. DeLoach, unpublished data).

4.3. Release of beetles adapted to the southwestern US

Diorhabda elongata beetles from all areas studied to date appear to be host specific to the genus *Tamarix* when considering the combination of host-plant larval suitability and acceptability to adults. Of the southern-adapted populations assessed, i.e., originating from below 43°N latitude in the Old World, none should pose any risk to plants outside the order Tamaricales and only a low risk to the native species of *Frankenia*, with *F. salina* potentially being most at risk of the species tested (but see Dudley and Kazmer, 2005). Uzbekistan beetles may pose a greater risk to athel compared to *D. elongata* from Crete, Tunisia or Turpan, However, this question needs to be resolved further, especially for Mexican officials. Saltcedar has invaded large areas in northern Mexico, where it is damaging natural areas and contributing to the acute water shortages along the Río Bravo and in other areas. Athel trees are also grown in northern Mexico and are valued to some degree as shade trees, hedges and windbreaks. Because all the beetle populations are expected to damage athel to some extent, approval of Mexican scientists, natural areas managers and authorities is being sought before releases are made along the Rio Grande, Texas. Ultimately, the decision to release any biological control agent is a compromise between minimizing the known damage being inflicted by the weed and the risk of non-target damage by the agent. In the present case, the predicted risk that *D. elongata* may pose to *Frankenia* or athel has to be thoughtfully balanced against the many known negative impacts (DeLoach et al., 2000) that invasive saltcedars have on riparian ecosystems in North America.

Acknowledgments

We express our sincere appreciation to our colleagues who collected the beetles—Rouhollah Sobhian, Alan Kirk (USDA-ARS European Biological Control Laboratory, Montferrier-sur-Lez, France), Javid Kashefi (USDA-ARS EBCL, Thessaloniki, Greece), Massimo Cristofaro (ENEA C.R. Casaccia, Rome, Italy), Baoping Li (Nanjing Agricultural University, Nanjing, China) and Ray Carruthers (USDA-ARS, Albany, CA). We thank James Tracy, Tom Robbins, Tanya Wickman, and Cari LaCaille (USDA-ARS, Temple, TX) for technical assistance; Dan Bean (University of California-Davis), Tammy Wang, and David Laclergue (USDA-ARS, Albany, CA) for supplying beetles; John Gaskin (USDA-ARS, Sidney, MT) for DNA analysis of *Tamarix* species; Joaquin Sanabria (Texas Agricultural Experiment Station, Temple, TX) for statistical advice; John Herr (USDA-ARS, Albany, CA) for experimental advice; Javier Betran, Bill Rooney, Mark Burow, Jackie Rudd (Texas Agricultural Experiment Station), and Anna McClung (USDA-ARS) for seed; and anonymous reviewers for their comments which improved this manuscript. This research was funded in part by a USDA-CSREES-IFAFS Grant #00-52103-9647.

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