

Abstracts – Session 3: Biological Control

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Effects of exotic plant removal and fuels reduction on vertebrates along the Middle Rio Grande, New Mexico

Heather L. Bateman¹, Alice Chung-MacCoubrey¹, Deborah M. Finch¹, David Hawksworth¹

¹Biology Department, University of New Mexico, Albuquerque, NM hbateman@unm.edu, achungmaccoubrey@fs.fed.us, dfinch@fs.fed.us, dhawksworth@fs.fed.us

Abstract:

Riparian cottonwood forests of the Middle Rio Grande have been severely invaded by exotic tree species, which, in combination with large accumulations of woody debris, make these forests prone to catastrophic, stand-reducing fires. Land managers need effective methods that eliminate fuel loads and invasive plants with minimal impacts on native vegetation and wildlife. As a component of a larger study, this project is evaluating the effects of fuels reduction and invasive plant removal on herpetofauna, avifauna, and bat communities. Here we present preliminary analyses of the first 6 years of this study. In 2000, we established 12 study sites and a randomized block design (4 treatments, 3 replicates). Pre-treatment data were collected from 2000-2003, and post-treatment data were collected from 2003-2006. We used pitfall and funnel traps to monitor reptile and amphibian populations, Anabat detection systems to monitor bat activity, and point count stations to estimate abundance and density of birds. The reptile and amphibian community was dominated by upland species rather than riparian obligates, and species composition did not change significantly after treatment. Based on principal components and regression analysis, 5 of 6 lizard species were less abundant in dense, cluttered, and invaded understories or more abundant in habitats with few exotics. Restoration treatments do not appear to have a negative impact on existing lizard populations. Using minutes of recorded bat activity as an index of relative site use, we determined that treatments increased summer bat activity (more minutes of activity) relative to untreated sites. Birds were placed into four nesting guilds. Overall bird species richness did not change in response to treatment; however there were species-specific responses to treatments. Three mid-story nesting species and one ground-shrub nesting species showed declining abundance from treatments, whereas cavity nesting and canopy nesting species were not affected by treatments.

Diapause induction limits dispersal of *Diorhabda elongata*

Daniel W. Bean¹, Nina Louden¹, Allard A. Cossé², Robert J. Bartelt², Jerry Shue¹ and Brian Swedhin¹

¹Colorado Department of Agriculture, Biological Pest Control, Palisade Insectary, 750 37.8 Road, Palisade, Colorado 81526. dan.bean@ag.state.co.us, ninalouden@hotmail.com, shue.jerry@gmail.com, bswedhin@hotmail.com. ²USDA Agricultural Research Service National Center for Agricultural Utilization Research Crop Bioprotection Research Unit 1815 N. University Street Peoria, Illinois 61604. bartelrj@ncaur.usda.gov, cosseaa@ncaur.usda.gov

Abstract:

In this study we investigated the role of the developmental state of adult *Diorhabda elongata* (diapause or reproductive) in determining dispersal behaviors as well as dispersal-associated behaviors such as release of aggregation pheromones under laboratory conditions and response to pheromones and plant volatiles in the field.

The induction of reproductive diapause in *D. elongata* occurs under daylengths less than about 14 hr 40 min while most of the population will be reproductive at longer daylengths. This occurs in laboratory reared insects as well as in field populations. The developmental path of laboratory reared insects can be controlled by simple manipulation of photoperiod and in the field the

decrease of daylength in the summer induces hibernal diapause.

On live plants, under controlled temperature and photoperiod, adult beetles showed a significantly higher percentage of dispersal behaviors (flight and attempted flight) under long days (16L:8D) than under short days (12L:12D). Under long days the dispersal behaviors were significantly more frequent late in the day than in the morning. In addition the short day treated insects ceased feeding and moved from the plants to the leaf litter beneath the plants after about 15 days. Males emitted the aggregation pheromone blend at a rate approximately 100 times greater under long days than under short days. These laboratory based results suggested that dispersal, aggregation and mating would be dependent on long day photoperiods and thus developmental state in the field.

Dispersal and developmental state of *D. elongata* populations were monitored during two different years at two different field sites. First, at the Lovelock, NV release site beetles were monitored during the summer of 2003. Diapause was induced in early August and the expansion of the population slowed and ceased by August 25. At two field sites on the Colorado River the rate of tamarisk defoliation and the movement of beetles were greatest in July and early August of 2006. By mid August the beetles had reached the full extent of their summer dispersal, defoliation slowed, and most of the population entered diapause.

Diapause-destined beetles still flew from defoliated areas to nearby areas that had green foliage. Using traps baited with green leaf volatiles or with pheromone we found that the ecological context of diapause destined beetles dictated dramatically different responses to attractants. Adult beetles moving through defoliated areas were strongly attracted to plant volatiles and weakly attracted to the pheromone mixture. In areas adjacent to defoliated trees, where beetles were extremely abundant on green foliage, there was no attraction to either pheromones or green leaf volatiles.

From these results we conclude that reproductive adults are the main dispersal agents in this species. They show dispersal behavior even in the presence of green foliage and our observations indicate that they have at least two different dispersal modes; short distance reproductive swarming and long distance flights. These may be related to population density or resource availability; this is an area for further investigation. As adults enter diapause dispersal is limited to the search for green foliage. Hungry adults are attracted to green leaf volatiles and when they find suitable foliage they remain there, feed and eventually drop to the leaf litter for overwintering.

These results add to an understanding of dispersal in *D. elongata*. The shift in behavior that comes with diapause can be incorporated into predictive dispersal models. Collection of large numbers of insects for biocontrol implementation will be aided by a better understanding of when and where aggregations may be found. ^aBean et al. 2006. Environ. Entomol. in press; ^bCossé et al. 2005. J. Chem. Ecol. 26:1735-1748; ^cCossé et al. 2006. J. Chem. Ecol. in press.

Pheromone and host odor attractants for managing *Diorhabda* spp.: Biological control agents of saltcedar

Allard A. Cossé¹, Robert J. Bartelt¹, Bruce W. Zilkowski¹, Nina Louden², Daniel W. Bean², Earl R. Andress³

¹USDA Agricultural Research Service National Center for Agricultural Utilization Research Crop Bioprotection Research Unit 1815 N. University Street Peoria, Illinois 61604.

cosseaa@ncaur.usda.gov, bartelrj@ncaur.usda.gov, zilkowbw@ncaur.usda.gov. ²Colorado Department of Agriculture, Biological Pest Control, Palisade Insectary, 750 37.8 Road, Palisade,

Colorado 81526. Dan.Bean@AG.STATE.CO.US, ninalouden@hotmail.com. 3USDA APHIS 4151 Hwy 86 Bldg 10 Brawley CA 92227. Earl.R.Andress@aphis.usda.gov

Abstract:

The recent identification of the aggregation pheromone^a and host odor attractant^b for the leaf beetle *Diorhabda elongata*, a biological control agent of saltcedar, allowed for a more detailed study of the chemical ecology of this beetle. Field results have shown that male and female *D. elongata* are clearly attracted to the two component pheromone. Additional lab studies have shown that the different *Diorhabda* strains, adapted for release at variable US latitudes, have identical pheromone compositions, but differ in component ratios.

The host odor attractant mimics the release of some antennally active green leaf volatiles that are emitted by saltcedar while being fed upon by adults and larvae. The green leaf compounds currently used in the host odor baits are present in emissions from all *Tamarix* species that are targeted for biological control and these baits are highly attractive to the beetles throughout the season. Furthermore, trapping data showed that combining the pheromone and host odor baits attracted even higher numbers of beetles and this combination showed a strong synergistic effect.

The attractants can be useful tools in the overall biological control program of saltcedar. The attractive baits have been successfully used in monitoring the presence of newly released beetles, in areas such as Lovelock, NV, and Moab, UT. Beetles dispersal from well established populations can be easily followed by baited traps, even in areas with no visual proof of any beetle presence. Also, retaining newly released beetles at the sites of release with the use of both pheromone and host odor has shown promising results, and this technique might be helpful in increasing the success rates of population establishment.

Lab and field work has demonstrated differences in biological activity of the single constituents that make up a behaviorally attractive blend, and these results are aiding our aspirations in trying to understanding the complex chemical beetle-plant interactions and mechanisms of beetle colonization of uninfested saltcedar.

^aCossé et al. 2005. J. Chem. Ecol. 26:1735-1748; ^bCossé et al. 2006. J. Chem. Ecol. in press.

Regional testing of *Diorhabda 'elongata'* ecotypes

P. Dalin¹, T. Dudley¹, D.C. Thompson², D.W. Bean³, D. Eberts⁴, D. Kazmer⁵, J. Michels⁶, P. Moran⁷, J. Milan⁸, C.J. DeLoach⁹

¹Marine Science Institute, University of California, Santa Barbara. dalin@msi.ucsb.edu, tdudley@msi.ucsb.edu. ²New Mexico State University, Las Cruces. dathompson@nmsu.edu. ³Colorado Department of Agriculture, Palisade Insectary and University of California, Davis. dan.bean@ag.state.co.us. ⁴US Bureau of Reclamation, Denver. deberts@do.usbr.gov. ⁵Agriculture Research Service, US Department of Agriculture, Sidney. dkazmer@sidney.ars.usda.gov. ⁶Texas Agricultural Experiment Station, Bushland. asychis@aol.com. ⁷Agriculture Research Service, US Department of Agriculture, Weslaco. pmoran@weslaco.ars.usda.gov. ⁸Bureau of Land Management, US Department of the Interior, Boise. Joseph_Milan@blm.gov. ⁹Agriculture Research Service, US Department of Agriculture, Temple. jdeloach@spa.ars.usda.gov

Abstract:

The Eurasian saltcedar leaf beetle *Diorhabda elongata* has been introduced into several western states for the biocontrol of tamarisk. Establishment was successful at some sites, with heavy defoliation and subsequent mortality of plants observed at sites in northern Nevada. However, at sites south of 37-38° N, the original form of *D. elongata* (collected in Fukang, China 44.1° N) failed to establish. Incubator studies indicated that failure was because this ecotype

responds to declining daylength by entering reproductive diapause too early in the season to successfully overwinter.

The purpose of the present study is to test different ecotypes of *D. elongata*, currently held under quarantine in the US (China I 44.1° N, China II 43.5°, Uzbekistan 38.1°, Greece 35.1° and Tunisia 34.4°), inside double secure cages in the field at sites ranging from 33-48° N in latitude. Our predictions are: 1.) Beetles will be reproductively active for the longest time period at those latitudes that match their latitudes of origin, and, 2.) Beetles will have the highest over-wintering survival at matching latitudes. Regulatory concerns that *D. elongata* ecotypes pose risks to the native salt-marsh plant *Frankenia salina* have delayed this research, but we were able to initiate the study in August of this year. Observations do, however, suggest that taking advantage of the full range of beetles might lead to increased success of the biocontrol program. At one southern site in Texas (31.5° N), the Crete ecotype has established with substantial population growth in 2006. The current experiment will provide crucial knowledge about how to promote biocontrol of tamarisk over the widespread range of infestations in western United States.

Preliminary success in biological control of saltcedar -Texas/New Mexico

C.J. DeLoach¹, A.E. Knutson², P.J. Moran³, D. Eberts⁴, G.J. Michels⁵, M. Muegge⁶, D.C. Thompson⁷, D. Richman⁷, J. Sanabria⁸, J.H. Everitt⁹, V. Carney⁵, K. Gardner⁷, J.L. Tracy¹, T.O. Robbins¹, J. Hudgeons² and R.I. Carruthers¹⁰

¹USDA-ARS, Grassland, Soil and Water Research Laboratory, Temple, TX. jdeloach@spa.ars.usda.gov. ²Texas A&M University Research & Extension Center, Dallas, TX. a-knutson@tamu.edu. ³USDA-ARS, Kika de la Garza Subtropical Agricultural Research Center, Beneficial Insects Research Unit, Weslaco, TX. pmoran@weslaco.ars.usda.gov. ⁴USDI-Bureau of Reclamation, Denver, CO. deberts@do.usbr.gov. ⁵Texas A&M Experiment Station, Bushland, TX. asychis@aol.com. ⁶Texas A&M University, Texas Agricultural Extension Service, Ft. Stockton, TX. ma-muegge@tamu.edu. ⁷New Mexico State University, Department of Entomology, Plant Pathology and Weed Sciences, Las Cruces, NM. dathomps@nmsu.edu; nmbugman@taipan.nmsu.edu. ⁸Texas Agricultural Experiment Station, Temple, TX. sanabria@brc.tamus.edu. ⁹USDA-ARS, Kika de la Garza Subtropical Agricultural Research Center, Integrated Farming & Natural Resources Research Unit, Weslaco, TX. jeveritt@weslaco.ars.usda.gov. ¹⁰USDA-ARS, Exotic and Invasive Weeds Research Unit, Albany, CA. ric@pw.usda.gov

Abstract:

Biological control has successfully controlled 13 exotic, invasive weeds of rangelands and natural ecosystems in the United States since 1945, control of others is in progress, and many more have been controlled worldwide. We initiated biological control of saltcedars (*Tamarix* spp.) at Temple, TX in 1986, using host-specific insect herbivores that regulate saltcedar populations in the Old World. Our cooperators in France, Israel, Kazakhstan, China and Turkmenistan tested 20 candidate control insects. Then, after quarantine testing at Temple, and being joined by the new ARS-EIWR Unit at Albany, CA (led by Carruthers) we released the leaf beetle, *Diorhabda* sp. (Coleoptera: Chrysomelidae) from Fukang, China and Chilik, Kazakhstan, into the open environment at 10 sites in May 2001. They established at 5 of the 6 northern sites and by August 2006 had defoliated approximately 85,000 acres at Lovelock, NV, 30,000A at Schurz, NV and Delta, UT, 8,000A at Lovell, WY and 300A at Pueblo, CO (discussed by others at this symposium).

These northern beetles failed to overwinter or to establish in Texas and southern California because the short summer daylengths cause premature overwintering diapause. In 2002, our

overseas cooperators sent *Diorhabda* ecotypes from 4 more southern latitudes. In tests at Temple and Albany, these beetles severely attacked the most widespread and damaging saltcedars, attacked other *Tamarix* spp. less, and were safe for all other 60 plant species tested. We released the Crete/Posidi, Greece ecotype into field cages and then into the open environment at 9 sites in Texas, 3 in New Mexico, and 2 in California from 2003 to 2005. We now have overwintering, establishment and rapid increase at Big Spring, and Lake Merideth, TX and probably also near Artesia, NM. At Big Spring, we released 38 adult Crete beetles in late April 2004 which defoliated 2 small trees by July; we added another 500 beetles in July which together with those defoliated another large tree by late September. In 2005, these increased rapidly and defoliated an area of 1.6A (210 large and medium-sized trees) by mid September. By late August 2006, they had defoliated an area of 10A. We have redistributed these beetles to several sites in the Big Spring/Sweetwater area and at Ft. Stockton, TX during 2005-6 and studied best methods for release. In uncaged, open field tests in southern Texas near Kingsville and Encino, and at Big Spring and Ft. Stockton, we are comparing the effects of the Crete and Tunisia beetles on saltcedar vs. the exotic athel (*Tamarix aphylla*) trees in preparation for consultations with Mexico regarding releases along the Rio Grande. Athel has some beneficial values for shade trees and windbreaks in the southern U.S. and northern Mexico. At Lake Merideth, TX we released the Posidi, Greece and Uzbek ecotypes in 2005 which now are established, dispersing and defoliating saltcedar. Near Artesia, NM, we released the Crete ecotype in August 2003 but they failed to establish after initially overwintering and defoliating saltcedar. A release of the Fukang ecotype in field cages near Artesia in May 2005 continued to reproduce into October, overwintered well, and were released into the field in May 2006. These field populations have significantly increased, probably representing an adaptation to more southern areas with shorter daylength. Intensive monitoring is underway. Where established, these beetles are providing self-sustained, permanent, environmentally friendly, and low cost control of saltcedars that is safe to all native plant species. We expect this to allow recovery of native riparian plant communities where water tables and soil salinity are satisfactory, and to improve wildlife and fish habitat, reduce wildfires, increase availability of water, and increase recreational usage of parks and natural areas.

Effects of host condition and genotype on performance of *Diorhabda elongata* (Chrysomelidae)

Tom Dudley^{1,2}, Peter Dalin¹, Robert Pattison³, Dan W. Bean⁴, Andrea Caires⁵

¹Univ. of Calif., Santa Barbara. tdudley@msi.ucsb.edu, dalin@msi.ucsb.edu. ²Univ. of Nevada, Reno. ³USDA-ARS, Reno. rpattiso1@hotmail.com. ⁴Colorado Dept. of Agriculture, Palisade, CO. dan.bean@ag.state.co.us. ⁵Utah St. Univ. threepin10@yahoo.com

Abstract:

Herbivore behavior and performance can be related to host plant qualities that vary with growing conditions, nutrient availability, foliage age or prior experience, or genotype. Such variation across a broad geographic range may account for some of the mixed results seen following the introduction of the saltcedar leaf beetle *Diorhabda elongata* (sensu lato) for biocontrol of *Tamarix* spp. A series of field and greenhouse studies in California and Nevada showed how several factors alter insect responses, including: 1) nitrogen (NO₃) augmentation accelerated larval growth and time to pupation, although pupae were significantly smaller than insects on plants grown in control soil; 2) moderate increase in soil salinity had no significant effect on growth, but high levels (resulting in visible salt exudation) caused foliage to be avoided by larvae and adults; 3) in the field, plants defoliated the previous year were avoided by adult beetles early in the season; 4) prior defoliation reduced larval growth and survival in a field experiment but had no effect when defoliation was simulated with potted plants -tissue age probably caused control

(non-defoliated) plants to be a poorer resource; 5) larval growth was slightly better on *T. ramosissima* foliage than on *T. parviflora*, *T. aphylla* or a *T. aphylla* x *ramosissima* hybrid grown in pots; 6) under 'natural' field conditions, *T. parviflora* was avoided by adult insects; 7) oviposition on field transplants of the previous 4 genotypes was high on *T. ramosissima* and the *T. aphylla* x *ramosissima* but very low on *T. aphylla* and *T. parviflora*, while all genotypes close (< 2 m.) to defoliated 'natural' *T. ramosissima* were eaten by dispersing starved larvae but at greater distance oviposition alone led to defoliation of only the 'preferred' genotypes. Host factors, particularly host species, account for target plant avoidance and poor *Diorhabda* establishment at some sites, and may reduce biocontrol agent performance at others, so should be incorporated into introduction plans in order to better predict establishment success across the infested range of tamarisk/saltcedar.

Biological control of tamarisk: Establishment, population increase, and impacts of *Diorhabda* spp. at experimental release sites in the western US

D.J. Kazmer¹, C.J. DeLoach², D. Bean³, R.I. Carruthers⁴, T.L Dudley⁵, D. Eberts⁶, A.E. Knutson⁷, D.C. Thompson⁸

¹USDA-ARS, Northern Plains Agricultural Research Lab, 1500 N. Central Ave, Sidney, MT 59270. dkazmer@sidney.ars.usda.gov. ²USDA-ARS, Grassland, Soil and Water Research Lab, 808 E. Blackland Road, Temple, TX 76502. JDELOACH@spa.ars.usda.gov. ³Colorado Dept. Agriculture, Biological Pest Control Program, 750 37.8 Rd., Palisade, CO 81526. Dan.Bean@AG.STATE.CO.US. ⁴USDA-ARS, Exotic and Invasive Weeds Research Unit, 800 Buchanan St., Albany, CA 94710. ric@pw.usda.gov. ⁵Marine Science Institute, Univ. of California, Santa Barbara 93106-6150. dudley@msi.ucsb.edu. ⁶USDI-Bureau of Reclamation, P.O. Box 25007, D-8220, Denver, CO 80225. deberts@do.usbr.gov. ⁷Dept. of Entomology, Texas A&M University, Dallas, TX. a-knutson@tamu.edu. ⁸New Mexico State University, MSC 3BE, Box 30003, Skeen Hall N220, Las Cruces, NM 88003-8003. dathomps@nmsu.edu

Abstract:

An experimental release program for *Diorhabda elongata* sensu lato, a leaf beetle that is the first biological control agent for tamarisk, was approved in late 1998. The Fukang (China) and Chilik (Kazakhstan) ecotypes of *D. elongata* were first released in field cages starting in 1999 at 10 sites in California, Colorado, Nevada, Texas, Utah and Wyoming. Open field releases were first made in 2001 at 7 of the 10 sites. Additional experimental release sites have been added since 2001 in some of the latter states as well as Montana, New Mexico, and Oregon. Results to date have been extremely variable, ranging from failure of the beetles to establish viable populations outside of the field cages to spectacular outbreak populations that have defoliated tens of thousands of hectares of tamarisk. The long critical photoperiod for induction of reproductive diapause of the Fukang ecotype appears to have limited its ability to establish in southern locations. Ecotypes of *D. elongata* from Turpan (China), Greece, Uzbekistan and Tunisia have shorter critical photoperiods and some open field releases of 3 of these ecotypes in southern locations show signs of preliminary success. One northern California site where the Fukang ecotype failed to establish is dominated by *Tamarix parviflora*, a less suitable host plant of the Fukang ecotype. An alternate *D. elongata* ecotype from Crete, Greece appears to have established and increased in numbers at this site. Predation, by birds and a large suite of generalist arthropod predators, is also hypothesized to be a major cause of establishment failure or limited population growth. Research on new tamarisk biological control agents is focusing on species that are less susceptible to generalist predators. Where populations of the Fukang and Chilic ecotypes are established, increasing dramatically in numbers, and repeatedly defoliating the same tamarisk

plants, the impacts on tamarisk include increased branch mortality, decreased flower and seed production, and some instances of apparent whole-plant mortality. No direct impacts on nontarget plants by *D. elongata* have been observed at these locations.

Mapping tamarisk biocontrol monitoring sites and the expansion of *Diorhabda elongata* populations

Brian Swedhin^{1,2}, Levi Jamison¹, Tom L. Dudley², and Daniel W. Bean¹

¹Colorado Department of Agriculture, Biological Pest Control, Palisade Insectary, 750 37.8 Road, Palisade, Colorado 81526. bswedhin@hotmail.com, LRJAMISON@fortlewis.edu, dan.bean@ag.state.co.us. ²Marine Science Institute, University of California, Santa Barbara, CA 93106. tdudley@msi.ucsb.edu

Abstract:

Mapping and monitoring tamarisk at release sites for the tamarisk leaf beetle *Diorhabda elongata* as well as following population expansion after beetle release are critical components of the tamarisk biocontrol program. In this study we tested the feasibility of using relatively simple and low cost methods for routine mapping and monitoring of biocontrol releases. Beginning in 2006 the Colorado Department of Agriculture has used GPS and GIS to establish and monitor *Diorhabda elongata* release sites in Mesa County, CO and at Dinosaur National Monument. Hand-held GPS units containing mapping software, maps and aerial imagery were used to set up monitoring sites, store coordinates of marked trees and collect data throughout the season. In addition, we have used mapping software and aerial imagery to follow the dispersal of *D. elongata* in Nevada and Utah.

Release and monitoring sites were selected by first examining aerial photos, choosing a potential site or sites and selecting trees based on distance from the potential release site. Our protocol calls for monitoring 25 trees comprising a release tree, 12 trees within 100 meters of the release and 12 trees between 100 and 200 meters of the release. Trees were selected and coordinates were programmed into the hand-held units prior to going into the field, which allowed us to rapidly locate potential release and monitoring trees once in the field. This was possible, in part, because we were able to check the points with a downloaded aerial photo or DRG (topo map). The results were an even distribution and correct spacing of monitoring trees and increased efficiency of the process such that an entire site could be set up and initial monitoring done in less than 4 hours. Using a drop-down menu monitoring data, such as condition of the plant, presence and stage of the beetles and the presence of predators, were entered directly into the hand-held GPS units which simplified data collection and later storage and analysis.

GPA and GIS has proven useful in the fine scale mapping of monitoring sites. Stands of tamarisk as well as isolated individual trees were mapped at a monitoring site where tamarisk density is very heterogeneous, some areas having solid monocultures and other areas having more open and dispersed stands. The movement of beetle populations through these areas will provide information on host selection as a function of stand density.

Large scale dispersal of *D. elongata* was also mapped using GPS and GIS. Using previous observations the expansion of *D. elongata* from the Lovelock, NV site was mapped over a three year period. Two release sites near Moab, UT have experienced major expansions of *D. elongata* populations in 2005-2006 following initial releases in 2004. These dispersal events, as well as major defoliations that have accompanied beetle population expansion, were mapped. There were less than 2 acres of defoliated tamarisk at the end of 2005. By August 2nd, 2006 there were 109.02 acres defoliated, covering 4.09 river miles upstream of the primary release site, and the

defoliated area was still expanding. Final results for 2006 will be compiled in mid September and presented. These results will be used to predict the timing of entry of the beetles into Colorado from release sites on the Colorado River in Utah. We anticipate that *D. elongata* will enter Colorado through the Colorado and Dolores River corridors by mid summer, 2007.

Open field host choice test of *Diorhabda elongata* (Coleoptera: Chrysomelidae) in Northern California

Hillary Thomas¹

¹UC Davis. hqthomas@ucdavis.edu

Abstract:

USDA-ARS has proven successful in establishing an efficacious biological control agent against *Tamarix* spp. at multiple release sites across the Western United States. However, a well climatically matched population of the agent, *Diorhabda elongata* (Coleoptera: Chrysomelidae) has failed to establish at Northern California release sites. Past laboratory host range testing and observational field evidence suggest that *D. elongata* exhibits differential host preferences amongst several invasive species of *Tamarix* but the contribution of host choices to establishment failure has remained speculative. I conducted an open field host choice test in July, 2006 between the dominant invasive, *Tamarix ramosissima*, and the host species present at California release sites, *Tamarix parviflora*. Thirty marked adult beetles were released onto each of three treatments, (1) *T. parviflora* only (2) *T. ramosissima* only and (3) A mixed treatment with both host species conterminous. *D. elongata* showed marked ovipositional preference for *T. ramosissima* over *T. parviflora* ($F=6.57$, $df=2,10$, $p=.015$), with no significant difference between the mixed and *T. ramosissima* only treatment. Adult presence was also significantly higher on the mixed and *T. ramosissima* only treatments than on the *T. parviflora* only treatment (repeated measures MANOVA $-F=7.93$, $df=2,14$, $p=.005$). These results show that the presence of a preferred host plant can dramatically increase oviposition and adult presence over time, suggesting that differential host preferences could be contributing to establishment failure at Northern California release sites.