ABSTRACT Several sibling species of the leaf beetle *Diorhabda elongata* (Brullé) have been introduced into North America for the biocontrol of saltcedars (*Tamarix* spp.), but only one, *D. carinulata* (Desbrochers), has been extensively used in the field. The first open releases took place in 2001, and widespread defoliation occurred at sites infested by *Tamarix ramosissima*, *T. chinensis*, and their hybrid forms. The beetles failed, however, to establish at sites where other *Tamarix* species are targeted for control. In this study, we compared the preference and performance of three *Diorhabda* sibling species using adult choice and larval performance experiments on the two formally targeted *Tamarix* species: *T. ramosissima* and *T. parviflora*. In the adult choice experiment, a greater proportion of *D. carinulata* was found on *T. ramosissima* than on *T. parviflora*. For the other two sibling species, *D. elongata* (Brullé) and *D. carinata* (Faldermann), adults were found in similar proportions on the two host plants. In the larval performance experiment, larval growth and survival did not differ between *Tamarix* species for any *Diorhabda* type; however, *D. carinata* larval biomass was 35–50% greater than the other beetles regardless of host species. Based on the few adults of *D. carinulata* found on *T. parviflora* in the adult choice experiment, we do not recommend introducing this beetle at sites where *T. parviflora* is targeted for biological control. The species *D. carinata* seems especially promising for future release because its larvae gained substantially more biomass than the other beetles during the same time period on both *Tamarix* species.

KEY WORDS *Diorhabda elongata*, introduced species, host choice, larval performance, tamarisk biocontrol

Most phytophagous insects feed on a restricted range of host plant species. Female insects typically choose to oviposit on host plants that constitute the best food quality for their offspring, leading to a positive linkage between adult preference and larval performance (Singer et al. 1988, Rank et al. 1998, Doak et al. 2006). Some insects do, however, chose to feed and lay eggs on less suitable hosts (Agosta 2006, Gripenberg et al. 2007). One possible explanation for the discrepancy between adult choice and larval performance is the time constraint many female insects experience in finding suitable oviposition sites within a relatively short life span, resulting in a lowered discriminative oviposition strategy (Larsson and Eckborn 1995, Gripenberg et al. 2007). In addition, if the insects are exposed to novel plant species, the adults may not recognize the plant as food, although their offspring could potentially feed and survive on it (Thompson 1985b, Strong et al. 1994, Dalin and Björkman 2006). Thus, many insects avoid suitable host plants for feeding and oviposition because of evolutionary and ecological constraints, which limit the range of host plant species used by the insects (Bernays and Graham 1988, Thompson 1998a, Thompson 2005). The use of specialized insects is believed to minimize the risks of unintended nontarget effects to the native flora but may at the same time limit the range of potential target plant species to which the insects can be effective. Because invasive weeds may constitute several species and hybrids that are intended for biological control (LeJeune and Seastedt 2001, Gaskin and Schaal 2003), multiple biocontrol agents may be needed to control the full range of target plants. To control several related invasive plant species and their hybrids, biocontrol managers may face a trade-off between releasing multiple herbivores and using fewer agents with somewhat broader host
ranges that are capable of feeding on a group of target plants. An additional strategy is to examine variation within one type of biocontrol agent and screen for populations or closely related species with subtle differences in host plant utilization and choose those agents showing the greatest preference and performance for the specific plant species targeted for biological control.

The leaf beetle *Diorhabda elongata* (Brullé) (Coleoptera: Chrysomelidae) was introduced into North America for the biocontrol of *Tamarix* spp. The beetles were originally collected in central Asia and released at many sites across nine states starting in 2001 (Dudley et al. 2001, Lewis et al. 2003). The beetles were referred to as a subspecies of *D. elongata* (*D. e. deserticola*), but the taxonomy has recently been updated, and the central Asia beetles should currently be referred to as *D. carinulata* (Desbrochers) (Tracy and Robbins 2009). In this paper, we will follow this new taxonomy of *Diorhabda* species group. A variety of *Tamarix* species are highly invasive in riparian areas where they have numerous detrimental impacts to biodiversity and ecosystem functions (Shafroth et al. 2005). It has been estimated that between 1 and 1.6 million acres or more are infested by *Tamarix* spp. in the western United States (Zavaleta 2000). Most of the *Tamarix* distribution is comprised of novel hybrids between the two species *T. ramosissima* and *T. chinensis*, which do not overlap in their native Eurasian ranges (Gaskin and Schaal 2002). Another *Tamarix* species invasive especially in central California in *T. parviflora* (Dudley et al. 2006). Establishment of *D. carinulata* was successful at several sites above 37°N in latitude in areas infested by the *T. ramosissima × T. chinensis* hybrids (hereafter referred to as *T. ramosissima*). The lack of establishment below 37°N in latitude is mostly explained by a developmental mismatch related to daylight and diapause induction in *D. carinulata* (Bean et al. 2007). Establishment failures also occurred at northern sites where the invasive *Tamarix* form was *T. parviflora*. Observations from the field where *T. parviflora* grows together with *T. ramosissima* suggest that *D. carinulata* avoids *T. parviflora* (T.D., unpublished data) and may therefore be an inappropriate agent for the biocontrol of *T. parviflora*. Other agents are needed to prevent the spread of this invasive plant.

To overcome the establishment limitations of *D. carinulata* at southern latitudes and at sites infested by *T. parviflora*, several other geographic forms of *D. elongata* were introduced into the United States (Milbrath et al. 2008). These beetles differ in several ecological aspects, especially in their response to temperature and daylight for diapause induction (Dudley et al. 2006). The beetles are considered to be different *Diorhabda* sibling species and colonies currently maintained under quarantine in the United States include *D. carinulata* from central Asia, *D. elongata* (Brullé) from the eastern Mediterranean area, and *D. carinata* (Faldermann) from western Asia (Tracy and Robbins 2009). All are specialized to feed on *Tamarix* spp. (Milbrath and DeLoach 2006), but there is limited information about what species of *Tamarix* the different sibling species feed on in their native ranges. The Mediterranean *D. elongata* may be adapted to feed on *T. parviflora* because its distribution range overlaps with the plant *T. parviflora* in the Mediterranean basin (Tracy and Robbins 2009). However, because of a lack of knowledge about the beetles’ host ranges, it is currently difficult to predict the success of the beetles in the control of *T. parviflora*.

The purpose of this study was to compare adult choice and larval performance on *T. ramosissima* and *T. parviflora* among three *Diorhabda* sibling species. The results from the experiments were expected to indicate which beetle type may be able to establish on *T. parviflora* and provide effective biocontrol of this invasive plant. Based on field observations suggesting that *D. carinulata* avoids *T. parviflora*, our primary hypothesis was that *T. ramosissima* is a better and preferred host for all three sibling species. When given a choice between the two host plant species, we predicted that a greater proportion of adult beetles would be found on *T. ramosissima*. We also predicted that larval performance (growth and survival) would be greater on *T. ramosissima*. An alternative hypothesis was that sibling species that are sympatric with certain *Tamarix* species in their areas of origin (e.g., *D. elongata* with *T. parviflora*; *D. carinulata* and *D. carinata* with *T. ramosissima*) would choose and show greatest larval performance on their presumably co-evolved host plant species.

**Materials and Methods**

**Adult Choice Experiment.** To study whether the three *Diorhabda* sibling species (*carinulata*, *elongata*, and *carinata*) prefer *T. ramosissima* over *T. parviflora*, we conducted a host choice experiment using adult insects at the University of California at Santa Barbara (UCSB) in August 2007. *D. carinulata* had originally been collected near Fukang, Xinjiang province, China (44°10’N, 87°59’E), *D. elongata* near Sfakaki, Crete, Greece (35°53’N, 24°60’E), and *D. carinata* had been collected near Karshi (Qarshi), Uzbekistan (38°86’N, 65°2’E) (Lewis et al. 2003, Milbrath et al. 2008). Colonies were maintained at the Palisade Insectary, Colorado State Department of Agriculture, Palisade, CO. We used adult beetles that had been grown from first instars on a mixture of *T. parviflora* and *T. ramosissima* foliage inside plastic containers in a laboratory environment at UCSB. We used both types of *Tamarix* for larval rearing to avoid beetles being conditioned to either of the two host plants. Larvae were provided fresh foliage every second day until pupation, when they were transferred into smaller containers with sand in the bottom for pupation.

For experimental arenas, we used 61 by 61 by 61-cm aluminum screen cages (Catalog 1450TC; BioQuip, Rancho Dominguez, CA) with one bouquet of *T. parviflora* and *T. ramosissima*, respectively, located at opposite corners in the cages. The plant bouquets were made from newly collected shoots (30–40 cm long) of *T. parviflora* and *T. ramosissima* trees culti-
vated in a common garden plot near UCSB. Thus, any differences in plant quality should have been owing to inherent traits of the plants rather than different growing conditions. The shoots were placed in glass vials with water and wrapped with plastic film around the vial opening to prevent the beetles from falling into the water. We used shoots with similar lengths and foliage density to avoid beetles discriminating between the two plant species based on structural differences. At the center of the cage, in between and at equal distances from the two bouquets, we placed a paper cup filled with sand containing one ~15-cm-long shoot of an ornamental plant species [Westringa fruticosa (Willd.); Lamiaceae] collected at the UCSB campus. This nonhost plant provided a climbing and flight initiation substrate for the beetles.

For each replicate, we released 10 newly emerged adult beetles onto the sand in the paper cup at equal distances from the two plant species bouquets. The experiment was conducted in a sunny, wind-protected, outdoor area at the UCSB campus. After 48 h, we counted the number of beetles on the two plant bouquets. Each Diorhabda sibling species was replicated nine times, and \( \chi^2 \) tests were used to compare the proportion of adult beetles on the two Tamarix species.

**Larval Performance Experiment.** To compare the quality of *T. ramosissima* and *T. parviflora* for larval growth and survival, we reared larvae enclosed in nylon mesh sleeves from newly hatched first instars to late third instars on plants under common garden field conditions. At the common garden, we used plants from three source populations for each of the two Tamarix species. The *T. parviflora* source populations originated from Woodland (CA), Tehachapi (CA), and Schurz (NV), and *T. ramosissima* originated from Bishop (CA), Barstow (CA), and Ventura (CA). The purpose of including several source populations per *Tamarix* species was to control for within-species variation in host plant quality for the beetles. Ten individual plants from each of the six source populations were used in the experiment (60 plant individuals in total). Because of a shortage of larvae for the sibling species *D. elongata*, we were only able to replicate this species twice on each plant source population, whereas the other two sibling species were replicated four times per plant source population. Plant individuals were assigned randomly to one of the three sibling species.

We obtained eggs of all three *Diorhabda* sibling species from the Palisade Insectary, Palisade, CO. The eggs were held under laboratory conditions at UCSB until hatching and ten larvae were transferred into each 30 by 70 cm nylon mesh sleeves placed on the *Tamarix* plants. Hatching time of eggs in the lab varied and the experiment was initiated over a three-day period starting on 15 July, 18 July, and 20 July in 2007. One nylon mesh sleeve was attached to each individual plant using wire twist ties to prevent larval escape and exclude predators. The sleeve was attached to the branch with the most foliage to minimize the risk of larval starvation during the study period. We also removed all predatory arthropods from the experimental branch before releasing larvae.

Larvae were left on the plants until 2 August 2007 when prepupae were first observed (i.e., large third instars moving actively inside the cage, ready to pupate). The experiment was terminated at this point because our intent was to compare maximum larval biomass attained. Insect larvae may lose weight when they cease feeding and initiate searching for pupation substrate (Waldbauer 1968). Our intent was to avoid such confounding influence on larval biomass. All experimental branches retained >50% of the foliage, indicating that larvae had a surplus of food throughout the study period.

The sleeves were brought back to the laboratory, and all surviving larvae were weighed individually to the nearest 0.001 mg (fresh weight) on an electronic balance. For statistical analysis, we used the mean terminal weight of individual larvae from each larval group as independent observations. Larval survival was calculated as the proportion of larvae remaining at the end of the experiment based on the initial number of insects. To test for differences between the two host plant species, we used “host plant species” and “*Diorhabda* sibling species” as fixed factors, and “host plant source population” as random nested factor (nested within “host plant species”) in two-way analysis of variance (ANOVA) (PROC GLM; SAS Institute 2008). The mean square for the nested factor was used as the error term when calculating \( F \) values for the fixed factor (Zar 1999). In addition, the three starting dates were treated as separate blocks in the analyses. Larval weight and larval survival data were normally distributed and generally met the assumptions for ANOVAs.

**Results**

**Adult Choice Experiment.** A greater proportion of adult beetles of *D. carinulata* were found on *T. ramosissima* than on *T. parviflora* (53 versus 14%; \( \chi^2 = 16.33; \text{df} = 1; P < 0.001 \)). The remaining proportion (33%) for *D. carinulata* were found on the starting substrate or on the cage wall. No significant differences in the proportions of adult beetles on *T. ramosissima* and *T. parviflora* were detected for the other two sibling species: *D. elongata* (50 versus 38%; \( \chi^2 = 1.25; \text{df} = 1; P > 0.10 \)) and *D. carinata* (47 versus 30%; \( \chi^2 = 2.77; \text{df} = 1; P > 0.05 \)).

**Larval Performance Experiment.** There were no significant differences related to host plant species in larval biomass (Table 1; Fig. 1A), nor larval survival (Table 1; Fig. 1B), for any of the *Diorhabda* sibling species. There was, however, significant variation in final biomass among the three species (Table 1). Over the same period of time, the western Asian *D. carinata* gained roughly 35–50% more biomass than the other two sibling species (Fig. 1A). There was no significant interaction between host plant species and beetle species in the analyses (Table 1), indicating that this growth response was independent of host plant species (i.e., *D. carinata* gained more weight regardless of what *Tamarix* species it was feeding on). Larval survival was statistically equal for all three *D. elongata* sibling species (Table 1). There was a trend for an effect of host plant source population on
larval biomass ($P = 0.072$ in Table 1), suggesting that variation in host plant quality among *Tamarix* genotypes may be larger than an overall difference between the two species *D. parviflora* and *T. ramosissima* for the *Diorhabda* species group.

### Discussion

The results from the adult choice experiment suggest that the central Asia beetle *D. carinulata* prefers *T. ramosissima* over *D. parviflora*, although larval performance (growth and survival) was equal on the two host plant species. For the other two sibling species (*D. elongata* from eastern Mediterranean and *D. carinata* from western Asia), the proportions of adult beetles were similar on the two plant species in the adult choice experiment, and larval performances were statistically the same on *T. ramosissima* and *T. parviflora*. This suggests that *D. elongata* and *D. carinata*, both of which have been released on a trial basis at selected sites in North America, may be better suited for the biological control of *T. parviflora* than is *D. carinulata*. The latter is the only sibling species that has been widely released in the implementation phase of the *Tamarix* biocontrol program (Lewis et al. 2003). Most of the *Tamarix* invasions in the United States are comprised of hybrids between *T. ramosissima* and *T. chinensis* (Gaskin and Schaal 2002), and *D. carinulata* has proven promising in the biocontrol of these highly invasive plants at sites above 37°N in latitude (Bean et al. 2007). Establishment of *D. carinulata* has, however, been unsuccessful at sites infested by *T. parviflora* (Dudley et al. 2006). Our results suggest that other sibling species of *D. elongata* may be better suited for use against *T. parviflora*.

The lack of significant differences in growth and survival of larvae of all three *Diorhabda* sibling species fed on *T. ramosissima* or *T. parviflora* indicates that these plant types are essentially equal as food resources for the beetles. Thus, we did not find any support for our hypothesis that *T. parviflora* should be a poor host. Neither did we find any strong support for our alternative hypothesis that beetles should show the highest preference for, and the best performance on, the host species that predominate in the beetles’ areas of origin. For example, we did not find an increased preference for or greater performance on *T. parviflora* in *D. elongata*, although the beetle’s distribution range overlaps with *T. parviflora* in the Mediterranean region where *T. ramosissima* is absent (Baum 1978, Tracy and Robbins 2009). Similar results were found for *D. carinata*, although the distribution ranges of the two *Tamarix* species suggest that this subspecies should be better adapted to feed on *T. ramosissima* (Baum 1978, Gaskin and Schaal 2002). The only result that seemed to support the alternative hypothesis was that *D. carinulata* from central Asia chose *T. ramosissima* over *T. parviflora* in the adult choice experiment. The avoidance of *T. parviflora* in *D. carinulata* adults may be explained by a lack of association between these two species in their native ranges.

Herbivorous insects can often extend their host range and include new plant species in their feeding range when exposed to novel hosts, especially if the plant is taxonomically related to the insects’ original hosts (Strong et al. 1994, Dalin and Björkman 2006, Roques et al. 2006). Specialized insects, like the ones studied here, are often highly sensitive to plant cues (e.g., plant volatile compounds) and discriminate among suitable host plants faster than generalist insects (Bernays and Funk 1999, Bernays 2001).

To include a new plant species in their feeding range, it is necessary that the adults are able to recognize the plant as food. Chrysomelid beetles
use a combination of visual, olfactory, and contact cues for host plant selection (Fernandez and Hilker 2007). Previous studies have indicated minor differences in growth performance of D. carinulata on T. ramosissima and T. parviflora with slightly slower growth rate on T. parviflora but that reduced field utilization of T. parviflora was most likely caused by adult avoidance (T.D., unpublished data). The results from our adult choice experiment confirm that D. carinulata adults may avoid T. parviflora. The underlying reason, or reasons, for adult avoidance of T. parviflora in D. carinulata remain unclear. The leaf beetle D. carinulata is attracted to a blend of green leaf volatiles emitted from T. ramosissima (Cossé et al. 2006), but these compounds are found in most green plants and are unlikely to provide sufficient means to distinguish between T. ramosissima and T. parviflora. It is possible that T. parviflora is lacking some other important olfactory or visual cues that are necessary for host recognition in D. carinulata adults. It is also possible that T. parviflora possesses physical or chemical deterrents that prevent acceptance by adult beetles.

Adult plant choices are of primary importance in determining which plants will be used by the larval stages in most herbivorous insects. Clearly, avoidance by adult Diorhabda beetles will lead to decreased utilization by larvae and may allow T. parviflora to escape the major episodes of defoliation seen with T. ramosissima at established biocontrol sites. It is therefore cost-effective to release an agent that exhibits both high preference for and good performance on the target plant species because the risk of establishment failure is otherwise high. The results from the adult choice experiment indicate that the sibling species D. elongata and D. carinata may establish more effectively on T. parviflora. The current establishment of D. elongata in an area infested by T. parviflora in northern California supports this notion (Carruthers et al. 2006). This sibling species has recently been proposed for release in southern states of North America, and establishment seems to have occurred at some sites in Texas (Milbrath et al. 2008).

The results from the larval performance study indicate that D. carinata may be especially promising for future release against Tamarix, because larvae of this species gained substantially more biomass than the other two sibling species. This suggests that D. carinata was more efficient at converting plant material into body mass or that they were feeding more during the same time period. The growth response of D. carinata was also independent of host plant species, suggesting that this beetle type may be superior compared with the other two sibling species for the biocontrol of both T. ramosissima and T. parviflora. Further studies are needed to study the mechanisms responsible for superior growth performance of D. carinata (metabolic conversion efficiency or higher ingestion rates) and how this may affect plant damage and the biocontrol of Tamarix. Other factors, such as overwintering survival, phenology, and timing of diapause induction, are also important factors to consider when selecting the most suitable beetle type for release (Bean et al. 2007, Milbrath et al. 2008).

There are at least six invasive species of Tamarix present in the United States, T. ramosissima, T. chinensis, T. parviflora, T. aphylla, T. canariensis, and T. gallica, as well as hybrids between these species (Gaskin and Schaal 2002, 2003). This great diversity of invasive Tamarix genotypes poses a challenge for biological control. Target plant genotype must be an important factor when considering which sibling species of Diorhabda to use for biological control. Our results indicate that, even though T. ramosissima and T. parviflora may be equal food resources for larvae, adults of D. carinulata may avoid T. parviflora in the field. For this reason, D. elongata and D. carinata may be more effective against T. parviflora. The results also suggest that the colonies of Diorhabda currently maintained under quarantine in North America may be preadapted to feed on wide range of Tamarix species but that adult avoidance behaviors may prevent feeding and oviposition on some Tamarix species.

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