Influence of Nutrient Levels in *Tamarix* on *Diorhabda sublineata* (Coleoptera: Chrysomelidae) Survival and Fitness With Implications for Biological Control

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**ABSTRACT** Establishment of the saltcedar leaf beetle (*Diorhabda* spp.) has been unpredictable when caged or released in the field for saltcedar (*Tamarix* spp.) biocontrol. It has been observed that one caged tree might be voraciously fed upon by beetles while an adjacent tree in the cage is left untouched. We hypothesized that differences in the nutrient content of individual trees may explain this behavior. We evaluated survival, development rate, and egg production of beetles fed in the laboratory on saltcedar foliage from trees that had been grown under a range of fertilizer treatments. Tissue samples from the experimental trees and from the field were analyzed for percent nitrogen, phosphorus, and potassium. There was essentially no survival of beetle larvae fed foliage from saltcedar trees at nitrogen levels below 2.0%. At levels above 2.0% N, beetle larvae had corresponding increased survival rates and shorter development times. Multiple regression analyses indicated that nitrogen and phosphorus are important for larval survival and faster development rates. Higher levels of potassium were important for increased egg cluster production. The plant tissue analysis showed that the percentage of nitrogen in the experimental trees reflected the range of trees in the field and also that there is high variability within trees in the field. Our research indicates that if beetles are released on trees with poor nutrient quality, the larvae will not survive.

**KEY WORDS** saltcedar, nitrogen, potassium, phosphorus, insect–plant interactions

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In 2001, saltcedar leaf beetles (*Diorhabda* spp.) were released in the western United States as a biological control agent for the invasive saltcedar tree (*Tamarix* spp.) (Dudley et al. 2001, Lewis et al. 2003, DeLoach et al. 2003, 2008). Observations of field cages used to establish beetles at a release site in southern New Mexico indicated that the beetles would feed voraciously on one saltcedar tree, but another tree within the same cage might not be fed upon at all, or the beetles would establish in one cage but not adjacent ones (Petersen 2007). We hypothesized that there could be many reasons for this phenomenon such as the presence of unpalatable hybrids (Gaskin and Schaal 2002), avoidance of drought-stressed trees (Dina and Klikoff 1973), differing phenolic levels in the leaf tissue (Coley et al. 1985), or differences in nutrient quality (Mattson 1980). We chose to focus on the possible role of nutrient differences for our experiments.

Few studies have been conducted regarding the nutrient requirements or composition of saltcedar (Badri and Hamed 2000, Marler et al. 2001) or the nutrient requirements for the saltcedar leaf beetle. In other plant–insect interaction studies, increased nitrogen levels in plant tissue often translates to increased survival and growth rates for insects (Wang et al. 2006, and studies reviewed in Mattson 1980, Scriber and Slansky 1981, Waring and Cobb 1992); however, some studies have shown no effect (Opit et al. 2005), or an increase in herbivore performance at lower or moderate levels of nutrients (Coetzee et al. 2007). At high levels of available nitrogen, toxic compounds that are nitrogen-based (such as alkaloids) may be produced in great quantities and can act as deterrents to herbivores, which would otherwise feed on the nitrogen-rich plants (Mattson 1980). Conversely, carbon-based defenses such as phenolics can decrease under high nitrogen conditions (Coley et al. 1985, Stout et al. 1998), and this may benefit herbivores.

Egg nutrient composition varies greatly among herbivorous insect species (Boggs 2009). The amount of time from when a female insect consumes nutrients to when those nutrients are incorporated into the egg structure can vary. In parasitic wasps, radioactively labeled amino acids were found in eggs laid from 2 hr to 13 d after ingestion (Rivero and Casas 1999). Nitrogen appears to be the major driving factor in insect development and reproduction, but phosphorus, potassium, and other elements play a role as well (Perkins et al. 2004, Walter and DiFonzo 2007).
In an effort to determine the influence of nutrient content in saltcedar on the establishment of the saltcedar leaf beetle, we tested the effects of a range of nutrient solutions on saltcedar trees grown in sand culture in the greenhouse and measured the consequent effects on the survival, development rate, and egg production of the beetle in the laboratory. We hypothesized that increased nutrient levels in the saltcedar tissue would correspond to increased larval survival, faster development rates, and higher egg production of saltcedar leaf beetles and therefore increase the likelihood of establishment in the field. This study also involved nutrient analysis of field-collected tissues to provide a comparison with the nutrient values of our experimental trees.

Materials and Methods

Greenhouse and Laboratory Experiment. Saltcedar stem cuttings (=50 cm in length) were removed from an individual saltcedar tree (Tamarix ramosissima Ledeb.) to ensure genetically identical test material. The cuttings were put into a container of aerated water and placed in a cooler at 4°C for 1 mo. The container was transferred to the greenhouse where the cuttings rooted over a 3-wk period (Wilkinson 1966). The plants were kept in the greenhouse at 24°C and a photoperiod of 14:10 (LD) h using supplemental lighting when necessary. The rooted stems were planted into 15.2-cm (one-gallon) pots with play sand that had been leached to clear the substrate of any nutrients. One hundred milliliters of a complete hydroponic solution (with the equivalent of 25 ppm N) was given to the plants three times a week to aid in establishing a root system.

After the experimental plants received the 25 ppm N fertilizer solution for 1 mo, 96 plants were assigned (in a randomized block design) to one of six blocks (located in the aforementioned greenhouse) and received one of four fertilizer treatments (Table 1). These fertilizer levels reflected values found in the field (Landenburger et al. 2006) and included extremes or levels that might be experienced with fertilization of trees in the field.

Four pots of each treatment were in each block. The different hydroponic fertilizer treatments were all dilutions from a hand-mixed stock fertilizer solution that contained all essential plant macronutrients and micronutrients (Table 1). The sources of nitrogen were potassium nitrate (KNO₃), 144 g potassium phosphate (KH₂PO₄), 251.5 g magnesium sulfate (MgSO₄·7H₂O), 1.4 g boric acid (H₃BO₃), 0.75 g manganese sulfate (MnSO₄·H₂O), 0.1 g zinc sulfate (ZnSO₄·7H₂O), 0.025 g copper sulfate (CuSO₄·5H₂O), and 0.015 g molybdenic acid (H₂MoO₄·7H₂O). After the saltcedar plants received the hydroponic treatments for 25 d, egg clusters from one cohort of our laboratory population of Diorhabda sublineata Lucas (origin: Tunisia, see Tracy and Robbins 2009) were divided into four groups and placed in Solo DMS 8-oz. clear plastic food cups with foliage from one of the four treatments. All of the cups for this experiment were held in the same rearing room at a photoperiod of 14:10 (LD) h at 28°C. There were 32 cups total from the experiments that contained variable numbers of clusters (usually three or four) depending on the availability from our laboratory population. Egg clusters for D. sublineata contained an average number of 12.3 (± 0.8 SEM) eggs. Three times per week, the hatched larvae were counted and carefully transferred to freshly harvested foliage from the respective treatment. The average number of larvae per cup was 44.6 (± 3.3 SEM), and overcrowding did not appear to be a problem based on ample food supply at the time of saltcedar changing. We harvested saltcedar foliage from one block in the greenhouse on each harvest day and rotated through the six blocks.

After larvae pupated (in sand substrate) and emerged as adults, they continued to be fed foliage from their respective treatment. A subset of adults from each treatment was randomly chosen and separated into male and female pairs. The number of adult pairs included in the experiment depended on survival and availability of saltcedar for that treatment. In total, 60 pairs of adults were included in the experiments. Each pair was placed into their own cup with saltcedar stems from their respective treatment level. The stems were inserted into wet floral foam for hydration. The number of egg clusters produced by the pair was

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final concn (ppm)</th>
<th>Ingredients in fertilizer solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>N P K</td>
<td>ml SS1</td>
<td>ml SS2</td>
</tr>
<tr>
<td>1 5 1.6 7</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>2 25 8 35</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>3 30 16 70</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>4 100 33 130</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>5 250 92 347</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>6 500 165 695</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

Four treatments were used in the first trial (treatments 1, 2, 4, and 5), and a separate set of four treatments (treatments 3, 4, 5, and 6) were used in the second and third trials.

a Stock solution 1: grams of ingredients for 1 liter = 251.5 g potassium nitrate (KNO₃), 144 g potassium phosphate (KH₂PO₄), 251.5 g magnesium sulfate (MgSO₄·7H₂O), 1.4 g boric acid (H₃BO₃), 0.75 g manganese sulfate (MnSO₄·H₂O), 0.1 g zinc sulfate (ZnSO₄·7H₂O), 0.025 g copper sulfate (CuSO₄·5H₂O), and 0.015 g molybdenic acid (H₂MoO₄·7H₂O).

b Stock solution 2: g ingredients for 1 liter = 539.5 grams of calcium nitrate Ca(NO₃)₂·4·H₂O, 24 g sequestrene 330 Fe, and 500 ml water.

Table 1. Final nutrient concentration (ppm N, P, K) and ingredients in each fertilizer solution applied to the experimental saltcedar (T. ramosissima) trees
recorded each time the foliage was changed (three times per week). The adult beetle portion of the experiment continued for a total of 30 d, until the death of the female, or until there was no longer adequate foliage to feed them.

The experiments were conducted from 3 April to 4 December 2009. Our first experiment included fertilizer treatments 1, 2, 4, and 5 (Table 1). After having no survival of larvae in the lowest two categories, we repeated the experiment two more times (using a new set of cuttings for these two experiments) and used fertilizer treatments 3, 4, 5, and 6. The results presented here include data pooled across all three replicate experiments.

The saltcedar plants in the greenhouse were tested for the percentage of nitrogen, potassium, and phosphorus in the foliage tissue at the beginning and end of each experiment. Leaf tissue was stripped from the stem and oven-dried. Approximately 5 grams of dried material from three blocks of each treatment were sent to A&L Plains Agricultural Laboratories, Inc., in Lubbock, TX for analysis. Nitrogen was determined by the Kjeldahl method, phosphorus was measured with a Gilford spectrophotometer, and potassium was determined using atomic absorption.

Field Tree Sampling. To compare our greenhouse plants to what occurs naturally in the field, we collected tissue samples from saltcedar trees at five different sites in southern New Mexico. Three sites were along the Rio Grande near Las Cruces, NM, approximately 1 km from each other. Saltcedar is often collected from these sites to feed our laboratory population of beetles. Additional sites were on the Pecos River near Lake Arthur, NM, and at an upland location on Holloman Air Force Base, 100 km northeast of Las Cruces. Saltcedar leaf beetles have been released at these latter two locations. At each site, we labeled and collected saltcedar tissue samples from five randomly chosen trees within 200 m of each other. We returned to the same trees once a month during May, June, July, and August of 2009 to collect tissue samples. Leaf tissue was taken from five random locations on the tree, stripped from the stems, placed in a paper bag, oven-dried, and sent to the aforementioned laboratory to be analyzed for the percentage of nitrogen, potassium, and phosphorus.

Data Analysis. Over the course of the experiment, the greenhouse saltcedar plants were given six different fertilizer solutions (Table 1). For the data analysis, we did not focus on the solution nutrient values, but rather used the values received from the tissue analysis to more accurately reflect what the nutrient levels were in the plants after uptake. The range of nutrient uptake values did not always reflect the direct gradient of the fertilizer solutions (Wang et al. 2006).

Analyses were conducted using SAS 9.2 (SAS Institute 2008). Tests for normal distributions were conducted on all data sets before analysis and were found to satisfy normality. The relationship between the larval survival, development rate (days to pupation), egg cluster production and nutrient levels (%N, P, and K) in the host plants was tested by linear regression. The sample sizes are different for each stage in the life history tested because of death at the extreme nutrient levels during the larval stage, where appropriate means were separated using pdiff in Proc GLM. Stepwise multiple regressions were performed (Proc Reg with model selection set to Stepwise) to analyze which nutrients in the tissue (%N, P, or K) at the beginning of the experiment most effectively explained the variation in larval survival and development rate. For egg cluster production, the nutrient values at the end of the experiment were also included in the model. The stepwise method allows variables (nutrient concentrations) to be added one by one to the model until the combination of variables that explain the most variation in the model are determined. A univariate repeated-measures approach was used for the analyses of samples from the same five field trees over 4 mo at five field sites. The main effect is site, the blocking factor is tree, and time is the repeated measures factor. Mauchly’s sphericity test was used to confirm the common covariance matrix of the transformed within-subject variables had variances and covariances equal to zero. Significance levels were set at $\alpha = 0.05$.

Results

Greenhouse and Laboratory Experiment. In 17 cases (number of cups) where the beginning nitrogen tissue levels were <2.0%, 1,277 out of 1,285 larvae died during the first or second instar (Fig. 1a). Survival was correlated with increases in the nitrogen content in the leaves ($r^2 = 0.838; F = 155.4; df = 1,31; P < 0.0001$; Fig. 1a). Larval survival was not correlated to phosphorus ($r^2 = 0.004; F = 0.14; df = 1,31; P = 0.716$; Fig. 1b) or potassium ($r^2 = 0.033; F = 1.01; df = 1,31; P = 0.322$; Fig. 1c) levels in the saltcedar tissues. The best model from the stepwise multiple regression analyses contained the values for beginning %N and beginning %P (df = 2, 29; $F = 103.6; P = <0.0001$; $R^2 = 0.877$). Beginning %N proved to be critical in larval survival (Table 2), and the beginning %P contributed to explaining a small amount of the variation.

Larvae that survived past second instar were used for the analysis of days to pupation (from date the egg cluster was laid to the first day of pupation). Development time was significantly faster in larvae fed saltcedar tissue with higher concentrations of nitrogen ($r^2 = 0.472; F = 12.5; df = 1,15; P = 0.003$; Fig. 2a) and phosphorus ($r^2 = 0.364; F = 8.0; df = 1,15; P = 0.013$; Fig. 2b). The best stepwise multiple regression model for days to pupation included all three parameters of beginning %N, %K, and %P in the tissue (df = 3, 12; $F = 61.5; P = <0.0001$; $R^2 = 0.939$; Table 3).

Because larvae fed saltcedar with <2.0% N did not survive to adulthood, we have no egg production data for beetles fed tissues with low nitrogen levels (<2.0%). At levels higher than 2.0% N, the average egg cluster production per female saltcedar leaf beetle per day was negatively correlated with nitrogen level ($r^2 = 0.080; F = 5.06; df = 1,59; P = 0.028$; Fig. 3a). There was however, significantly higher average egg
cluster production correlated with the higher levels of phosphorus ($r^2 = 0.139; F = 155.4; df = 1,31; P < 0.001; r^2 = 0.838$) and potassium ($r^2 = 0.272; F = 21.7; df = 1,59; P < 0.001$; Fig. 3b).

The best two-parameter model for average egg cluster production per female saltcedar leaf beetle per day contained the values for beginning %N and %K in the tissue ($df = 2, 40; F = 4.04; P = 0.0471; %K; df = 2,40; F = 3.25; P = 0.0332$). The beginning %N in the tissue yielded a negative coefficient for egg production. The ending tissue values did not contribute to the model.

Field Trees. Tissue analysis for the saltcedar foliage collected across southern New Mexico showed high variability between and within sites (Fig. 4). Repeated measures analysis of variance (ANOVA) indicated significant differences between sites for all nutrients tested (%N: $df = 4, 20; F = 7.72; P = 0.0006; %P: df = 4,20; F = 2.92; P = 0.0471; %K; df = 4,20; F = 3.25; P = 0.0332$). Nutrient levels changed over the season (%N: $df = 4,20; F = 2.40; P = 0.0452; %P: df = 4,20; F = 24.77; P < 0.0001; %K; df = 2,40; F = 57.87; P < 0.0001$); however, no significant interactions were found between site and sample date ($P > 0.05$). When focusing on just one site such as Holloman Air Force Base, the data indicated high variability in the nitrogen concentrations of the saltcedar.

Table 2. Results of a stepwise multiple regression analysis between saltcedar leaf beetle ($D. sublineata$) larval survival and beginning saltcedar ($T. ramosissima$) nutrient tissue values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>$F$</th>
<th>$P$</th>
<th>Partial $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1.33</td>
<td>75.78</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Beg %N</td>
<td>0.553</td>
<td>206.1</td>
<td>&lt;0.0001</td>
<td>0.038</td>
</tr>
<tr>
<td>Beg %P</td>
<td>1.702</td>
<td>9.22</td>
<td>0.0050</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Table 3. Results of a stepwise multiple regression analysis between days to pupation for saltcedar leaf beetles ($D. sublineata$) and beginning saltcedar ($T. ramosissima$) nutrient tissue values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>$F$</th>
<th>$P$</th>
<th>Partial $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>56.56</td>
<td>172.48</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Beg %N</td>
<td>-12.71</td>
<td>62.87</td>
<td>&lt;0.0001</td>
<td>0.472</td>
</tr>
<tr>
<td>Beg %K</td>
<td>21.50</td>
<td>72.92</td>
<td>&lt;0.0001</td>
<td>0.183</td>
</tr>
<tr>
<td>Beg %P</td>
<td>-143.03</td>
<td>55.65</td>
<td>&lt;0.0001</td>
<td>0.253</td>
</tr>
</tbody>
</table>

Fig. 1. Correlation of average larval survival of saltcedar leaf beetles ($D. sublineata$) with initial nutrient content in saltcedar ($T. ramosissima$) tissue used as host plants ($n = 32$).

Fig. 2. Correlation of average days to pupation of saltcedar leaf beetles ($D. sublineata$) with initial nutrient content in saltcedar ($T. ramosissima$) tissue used as host plants ($n = 16$).
tissue even between trees within 50 m of each other (ANOVA of average monthly nitrogen concentrations in the five trees: df = 4,15; F = 23.6; P = <0.001; n = 20; Fig. 5). The range of nitrogen found in our experimental greenhouse trees (1.7–3.2% N) accurately reflected the range of nitrogen found in the field (1.2–3.5% N); however, the field tree values for phosphorus and potassium were considerably lower (all <0.2% P and <1.4% K) than our experimental trees (0.11–0.36% P and 0.99–2.9% K).

Table 4. Results of a stepwise multiple regression analysis between average egg cluster production per female saltcedar leaf beetle (D. sublineata) per day and beginning and ending saltcedar (T. ramosissima) nutrient tissue values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>F</th>
<th>P</th>
<th>Partial R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.786</td>
<td>2.97</td>
<td>0.0901</td>
<td></td>
</tr>
<tr>
<td>Beg %K</td>
<td>0.540</td>
<td>24.21</td>
<td>&lt;0.0001</td>
<td>0.272</td>
</tr>
<tr>
<td>Beg %N</td>
<td>-0.364</td>
<td>7.70</td>
<td>0.0075</td>
<td>0.087</td>
</tr>
</tbody>
</table>

Discussion

The nitrogen content in the foliage of a saltcedar tree is a driving factor for the survival of saltcedar leaf beetle larvae feeding on that tree. Nitrogen tissue levels <2.0% resulted in virtually no survival of larvae.
past the second instar. A study involving chrysomelid beetles (*Chrysomela scripta* Fabricius, cottonwood leaf beetle) that feed on cottonwood (*Populus deltoides* W. Bartram ex Marshall) saplings with different nitrogen addition treatments also reported a threshold of 2.0% total leaf nitrogen in regards to feeding preference (Wait et al. 1998). The authors stated that *C. scripta* preferred to consume leaves with >2.0% leaf N, and consumed greater quantities of these leaves as well. Another study of a specialized chrysomelid beetle (*Paropsis atomaria* Olivier) feeding on *Eucalyptus* spp. L’Hér. indicated that the nitrogen content of the plants was more influential on the growth of the larvae than on the tannin levels (Fox and Macauley 1977). The *P. atomaria* larvae only had a net gain of nitrogen when feeding on plants above 0.9% N; this was important because the *Eucalyptus* foliage rarely contained >2.0% N.

Our finding has clear implications for the release of saltcedar leaf beetles. A common practice for establishing beetles in the field involves placing a cage around one or two saltcedar trees and releasing adults or larvae onto the caged trees (Hudgeons et al. 2007). This aids in keeping the beetles from dispersing too quickly. In these circumstances, it would be advantageous to test for nitrogen in the leaf tissue to ensure that it is sufficient to allow successful establishment, and if necessary, to fertilize the soil in the release cage, or in the immediate vicinity of the release area with a soluble nitrogen source such as urea to improve plant nutrition.

Higher values of nitrogen (>2.0%) and phosphorus (>0.02%) positively impacted the development rate of the saltcedar leaf beetles leading to a shorter number of days to pupation. This finding also has implications for release of beetles in the field. The faster a beetle population can reproduce, the greater the probability for successful establishment. This is especially true for saltcedar leaf beetles in the southern latitudes of saltcedar’s range in the United States where shorter summer day lengths can cause early diapause induction for some *Diorhabda* spp. (Bean et al. 2007).

The role of phosphorus in terrestrial insect development does not appear to have been thoroughly investigated. Because phosphorus is a component of important cellular structures such as membrane phospholipids, ATP, DNA, and RNA it is assumed that if phosphorus is limiting, then this would have an effect on growth rate as was demonstrated in tobacco hornworm caterpillars (*Manduca sexta* (L.)) feeding on sacred thornapple (*Datura wrightii* Regel) (Perkins et al. 2004). The importance of beginning %P as a significant variable in our beetle survival and development time models indicates that it is important for the saltcedar leaf beetle as well.

The beginning %K values did not appear to play a large role in larval development but were a significant variable in egg production. There is a relationship between plant potassium levels and plant protein and starch production (Waring and Cobb 1992), and it is possible that this could benefit egg production. Nutrient concentration values at the end of the experiment did not influence the egg production models at all. This implies that the quality of foliage the larvae are raised on affects their performance later in life more than the foliage they would consume as adults.

Tissue nutrient data collected from saltcedar trees across southern New Mexico revealed that the nitrogen levels in our experimental trees covered the range of variability of nitrogen in the field trees including the release sites. However, all of the field trees had lower levels of phosphorus and potassium in their tissues than did our experimental trees. This may indicate that phosphorus and potassium are limiting in these systems. In examining N:P ratios, 32% of the field tree samples (n = 101) indicated a phosphorus limitation (Koerselman and Meuleman 1996). The high variability in nutrients found in the field adds evidence to the importance of testing nutrient levels of trees planned for use in caged releases, because even in very close proximity nutrients in the saltcedar trees can vary greatly (Fig. 5).

From our own experiences in southern New Mexico, it has been challenging to establish saltcedar leaf beetles in the field despite the release of thousands of beetles over several years. There are several hypotheses for this challenge: the shorter summer day lengths, which induce diapause in the beetles too early (Bean et al. 2007); and concerns that the trees are drought-stressed, have higher phenolic levels in the leaf tissue, are unpalatable hybrids, or are of poor nutrient quality. During a caged saltcedar tree study at a beetle release site near Artesia, NM, trees in close proximity to each other but with different phenotypes (green or bluish foliage) were tested for differences in genetics because the beetles appeared to prefer feeding on the green foliage. Molecular analysis of these saltcedar trees concluded that there was no correlation between beetle feeding preference and the plant genotype (Petersen 2007), thus indicating no genetic differences between the plants. However, recorded nitrogen values for individual trees near Artesia, NM, and at Holloman Air Force Base, New Mexico where beetles have been released, are often below 2.0% (Zens 2008). While our study did not examine phenolic levels or drought stress, it is likely that a combination of these factors along with low nutrient values have contributed to difficulties in beetle establishment.

Another possibility to increase the success at saltcedar leaf beetle biocontrol sites could be to fertilize the saltcedar trees before they are caged. Future studies will analyze whether this is a feasible option or if it truly could make a difference in the success of a release where local trees might be low in nutrients. Fertilization of trees in the field could have an effect because the discriminatory feeding behavior has only been exhibited in cages, and the beetles are able to overcome this phenomenon in the field when a selection of trees is available. Overall, our study has revealed the importance of considering plant nutrient quality for saltcedar biological control efforts.
Acknowledgments
We thank the staff of the High Containment Insect Facility (Quarantine Laboratory) at New Mexico State University for devotion to the maintenance of the saltcedar leaf beetle colonies and saltcedar plants. Funding for this project was provided by grants from Holloman Air Force Base and the New Mexico Agricultural Experiment Station, New Mexico State University, Las Cruces.

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Received 19 March 2010, accepted 1 November 2010.