Environmental Pollution 157 (2009) 384–391

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Effects of pollutant accumulation by the invasive weed saltcedar (*Tamarix ramosissima*) on the biological control agent *Diorhabda elongata* (Coleoptera: Chrysomelidae)

Mary A. Sorensen [a,*], David R. Parker [b], John T. Trumble [a]

[a] Department of Entomology, University of California, Riverside, CA 92521, United States
[b] Department of Environmental Science, University of California, Riverside, CA 92521, United States

The presence of Se, but not perchlorate, Mn, or Cr (VI), in foliage of the invasive weed saltcedar was shown to reduce growth of the biological control agent Diorhabda elongata*.*

ARTICLE INFO

Article history:
Received 9 April 2008
Received in revised form 13 August 2008
Accepted 2 October 2008

Keywords:
Hyperaccumulator
Elemental defense
Heavy metal
Invasive species
Tamarisk

ABSTRACT

Hydroponic greenhouse studies were used to investigate the effect of four anthropogenic pollutants (perchlorate (ClO_4^-), selenium (Se), manganese (Mn), and hexavalent chromium (Cr (VI))) on the biological control agent *Diorhabda elongata* Brullé. Contaminant concentrations were quantified for experimental *Tamarix ramosissima* Ledab. plants and *D. elongata* beetles. Growth of larvae was significantly reduced by Se contamination, but was not affected by the presence of perchlorate, Mn, or Cr (VI). All of the contaminants were transferred from plants to *D. elongata* beetles. Only Cr (VI) was accumulated at greater levels in beetles than in their food. Because *T. ramosissima* grows in disturbed areas, acquires salts readily, and utilizes groundwater, this plant is likely to accumulate anthropogenic pollutants in contaminated areas. This study is one of the first to investigate the potential of an anthropogenic pollutant to influence a weed biological control system.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

In 2004, *Diorhabda elongata* Brullé (Coleoptera: Chrysomelidae), originally collected in China, was released in the western United States for biological control of saltcedar (*Tamarix*). In the United States, invasive weeds in the genus *Tamarix* are estimated to occupy 650,000 hectares from northern Mexico to Montana and from Kansas to California (Shafroth et al., 2005). The worst of these species is considered to be *Tamarix ramosissima* (DeLoach et al., 2003).

Maturation of *D. elongata* from egg to reproductive adult takes approximately 40 days, with both larvae and adults feeding on saltcedar foliage. The insects have been released at many locations, including sites in California, Nevada, Colorado, Utah, Wyoming, and Texas (DeLoach et al., 2004). Although one defoliation will not kill a saltcedar tree; multiple defoliations over multiple seasons or years have been shown to kill even large, established plants (Shafroth et al., 2005), suggesting that the establishment of large, permanent *D. elongata* populations is necessary for saltcedar control.

Invasion by saltcedar is facilitated by the plant's ability to thrive in soils high in salts (Arndt et al., 2004). Saltcedar's common name refers to high concentrations of salts which are often excreted through glands in the leaves, forming a visible white coating on the foliage (Arndt et al., 2004). The composition of salts excreted through foliage is dependent on salts present in the root medium, indicating that ion uptake is not always selective (Thomson et al., 1969; Berry, 1970; Storey and Thomson, 1994). Therefore, we predict that saltcedar will acquire pollutants present in water or soil, such as perchlorate (ClO_4^-), selenium (Se), manganese (Mn), and hexavalent chromium (Cr (VI)). These four contaminants all occur as salts which, when dissolved in water, form ions available for uptake by plants. Additionally, perchlorate, Se, Mn, and Cr are all anthropogenic environmental contaminants with demonstrated potential for accumulation by plants and toxicity to insects.

Perchlorate has been documented in drinking and irrigation water in at least 20 of the United States (US EPA, 2004). Urbansky et al. (2000) found that *Tamarix* in the Las Vegas Wash (Clark Co., NV) contained from 5 to 300 mg kg^{-1} dry mass (dm) perchlorate. Sorensen et al. (2006) showed that at concentrations of 25 mg l^{-1} perchlorate caused mortality and delayed development of mosquito larvae. Thus, the levels occurring in *Tamarix* in the Las Vegas Wash could be predicted to cause reduced development or increased mortality of insects feeding on these plants.

* Corresponding author. Tel.: +1 951 827 4733; fax: +1 951 827 3086.
E-mail address: mary.sorensen@ucr.edu (M.A. Sorensen).

0269-7491/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2008.10.001

Selenium contamination is a major concern in the United States, particularly in the western United States, where 1.5 million acres of farmland is affected (Brown et al., 1999). Selenium is known to accumulate in a variety of plants, at levels up to 100 mg kg^{-1} in most plants, and over 1000 mg kg^{-1} dm in Se-adapted hyperaccumulator plants (White et al., 2007). Although Se is essential to insects in small quantities (Martin-Romero et al., 2001), the consumption of elevated levels of Se in food can cause mortality in insect herbivores ($\sim$40 mg Se kg^{-1}, Vickerman et al., 2002), insect detritivores (0.1–0.4 mg Se kg^{-1}, Jensen et al., 2005, 2006), and aquatic insects (1–10 mg Se l^{-1}, Jensen et al., 2007).

Magnesium and Cr (VI) also accumulate in some plant species. In Mn-contaminated areas plants may acquire levels of Mn reaching as high as 2000 mg kg^{-1} (Voorhees and Uresk, 1990). A consolidated source of information regarding the distribution of Mn pollution is unavailable, but the most common sources of Mn pollution are iron and steel production and power plants (ATSDR, 1997). Although small amounts of Mn are essential for insects (Trager, 1953), the metal has the potential to be toxic at high concentrations (1400 mg Mn kg^{-1}, Coleman et al., 2005). Similarly, Cr has been shown to accumulate in some plants to levels exceeding 2000 mg kg^{-1} (dm, Vajpayee et al., 1999). Chromium contamination is a significant worldwide problem (World Bank, 2002), including areas such as southern California where saltcedar is abundant (Los Angeles Regional Water Quality Control Board, 2004). Interestingly, even short exposures allow substantial accumulation in plants. For example, concentrations in cauliflower exceeded 2 mg Cr kg^{-1} (dm) when exposed for only 1 week to 1 mg l^{-1} Cr (VI) (Zayed et al., 1998). Hexavalent chromium has been found to negatively impact aquatic macroinvertebrates (Leslie et al., 1999), mosquito larvae at 0.16 mg l^{-1} Cr (VI) (Sorensen et al., 2006), and an insect detritivore at 500 mg Cr (VI) kg^{-1} (fresh mass (fm), Trumble and Jensen, 2004).

The objective of our research was to quantify *D. elongata* larval growth while feeding on *T. ramosissima* plants grown in the presence of perchlorate, Se, Mn, and Cr (VI). Concentrations of contaminants acquired by the saltcedar plants and transferred to beetles were also quantified. Although effects of pollutants on natural enemies such as parasitoids used for biological control has been studied in a number of systems (e.g. Gate et al., 1995; Kazimirova et al., 1997; Holton et al., 2003), this is one of the first studies to look at pollutant effects on a biological control agent for weeds. Knowledge of pollutant effects on weed biocontrol could be one important component in explaining the variable successes of weed biocontrol agents across time and space.

2. Methods

2.1. Plants used for bioassays

Saltcedar plants were grown from cuttings. Stems approximately 4 cm in diameter and 0.5 m long were cut from *T. ramosissima* stands growing in the Santa Ana River Wash at Martha McLean/Anza Narrows County Park, Riverside, California (37.0625°N, 95.677068°W). Cuttings were taken from plants growing in the same $\sim$30 m^2 area and randomly assigned to treatments to minimize the effect of any genetic variability between plants. Stems were stripped of foliage and individually placed in 500 ml wide-mouth flasks with Miracle Gro™ nutrient solution at half of the label's recommended concentration. Flasks were topped off with growing solution daily. Once per week, the growing solution was changed and flasks were cleaned to remove algal growth. Plants began to develop roots within $\sim$1 week, and experiments were begun approximately 6 weeks after cutting were started, when plants were approximately 0.012 m^3.

2.2. Beetle colony

D. elongata eggs of the Crete ecotype were obtained in June 2006 from the USDA facility in Albany, CA. The colony was maintained in the UC Riverside Insectary and Quarantine facility, in a greenhouse room with natural light supplemented with artificial light to obtain a light–dark cycle of 14 h light:10 h dark and a constant temperature of 24 °C. Larval beetles were housed in plastic containers (40 × 18 × 16 cm) with a layer ($\sim$3 cm) of fine sand for pupation, and provided with field-collected saltcedar foliage daily. Adult insects were kept in $\sim$1 m^3 screened enclosures and provided with saltcedar foliage daily for oviposition and as a food supply. Eggs were collected once per week and added to a clean larval container. Second-instar larvae were removed as needed for use in bioassays. Although contaminant concentrations were not measured in foliage used for the colony (colony foliage was collected from a different field site than those analyzed for pollutant concentrations), insects used for various bioassay treatments were fed the same food and therefore had the same exposure history, validating comparisons between control and treated insects.

2.3. Bioassays

Growing solution for 6-week-old saltcedar plants was replaced with treatment solution consisting of tap water for control plants or tap water plus pollutant. Perchlorate was added to bioassay containers as ammonium perchlorate (CAS# 7790-98-9, NH_4ClO_4, Aldrich Chemicals, Milwaukee, WI), and concentrations are reported here as concentration of the perchlorate anion (ClO_4^-). Selenium was added in the sodium selenate form (CAS# 13410-01-0, Na_2SeO_4, Sigma–Aldrich, St. Louis, MO), and is reported as concentration of elemental Se. Manganese was added as manganese (II) chloride tetrahydrate (CAS# 13446-34-9, $MnCl_2 \cdot 4H_2O$, 98%, Sigma–Aldrich, St. Louis, MO), and is reported as elemental Mn. Lastly, Cr treatments were created using chromium trioxide (CAS# 1333-82-0, CrO_3, 99.8%, Fisher Scientific, Pittsburgh, PA), and are reported here as concentration of elemental Cr.

Treatment concentrations were chosen to represent the high end of the range of concentrations of each pollutant reported from contaminated sites (100 mg perchlorate l^{-1}, US EPA, 2004; 2 mg Se l^{-1}, Seiler et al., 1999; 50 mg Mn l^{-1}, Howe et al., 2004; 2 mg Cr l^{-1}, Zayed and Terry, 2003) without causing visible damage to the plants (yellowing, wilting, drying of foliage). All treatments were replicated four times (four plants). Additional treatment solution was added daily to keep the flasks full for the remaining 2 weeks of the experiment, but the solution was not changed. Refilling rather than replacing treatment water simulated a closed body of water subject to cycles of evaporation and replenishment. Water samples were collected at least two times to detect changes in pollutant concentration due to evaporation, volatilization, and plant uptake. While perchlorate and Se became more concentrated over time, some Mn and Cr were lost from solution. Plants were exposed to treatment water for 1 week before beetles were added.

Four second-instar *D. elongata* larvae were weighed and individually caged on each plant. Cages were constructed from 1 cm thick foam, cut into 6 × 4 cm rectangles with 4 × 2 cm sections cut out of the middle. The cut-out sections were covered with a piece of clear plastic $\sim$0.11 mm thick. Two rectangles were then taped together to form an enclosure with interior dimensions of 4 × 2 × 2 cm. Cages were placed onto sprigs of living saltcedar plant with a single beetle larva in each cage and four cages on each plant (for a total of four beetles per plant). In most cases, there was enough foliage to last the larva for the weeklong experimental period, but if the larva depleted its food resources, the cage was moved to another area of the plant. After 1 week of exposure, beetles were collected, weighed, and preserved for chemical analysis, and foliage was collected.

2.4. Chemical analysis

2.4.1. Perchlorate

Plant foliage was processed and analyzed according to Seyfferth and Parker (2006). Five to 10 g of frozen foliage was combined with 30–70 ml of HPLC-grade water (Milli-Q Water System, Millipore Corporation, Bedford, MA), macerated in a blender for $\sim$3 min, mechanically shaken for 1 h, and centrifuged at $1.0 \times 10^5 g$ for 1 h. The supernatant was vacuum filtered through a 0.22 μm polyethersulfone filter (Millipore Corp., Bedford, MA) then passed through a pre-conditioned ENVI-18 solid-phase extraction (SPE) cartridge (Supelco, Bellefonte, PA) to remove organic materials. Perchlorate analysis was performed using ion-chromatography–electrospray ionization mass spectrometry (IC–ESI–MS, Dionex Corp., Bannockburn, IL).

Insect larvae were placed in plastic test tubes with 8 ml of HPLC-grade water (Milli-Q Water System, Millipore Corporation, Bedford, MA), macerated by hand using a glass stirring rod, then microwave digested (MARS 5 Closed Vessel, CEM Corp., Matthews, NC). The resulting slurry was vacuum filtered with a 0.22 μm polyethersulfone filter (Millipore Corp., Bedford, MA), then passed through a preconditioned ENVI-18 solid-phase extraction (SPE) cartridge (Supelco, Bellefonte, PA) to remove organic materials. Water samples were filtered and passed through SPE cartridges as described above. The resulting clear filtrates from the larval and water samples were analyzed using IC–ESI–MS, as described in Seyfferth and Parker (2006).

2.4.2. Selenium, chromium, and manganese

Plant samples were freeze-dried (Labconco Corp., Kansas City, MO) at −40 °C and −25 psi for 3 days and finely ground using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA, USA). A sub-sample of the resulting powder was mixed thoroughly with two parts HNO_3, two parts H_2O_2, and one part H_2O and microwave digested (Milward and Kuckner, 1989). The resulting mixture was filtered (0.22 μm polyethersulfone filter, Millipore Corp., Bedford, MA) and the clear filtrate retained for analysis. Insect samples were digested as described for plant samples with whole

insects digested individually. Water samples were filtered as described and diluted as necessary using HPLC-grade water. Selenium analysis was performed using hydride generation atomic absorption spectroscopy (HG-AAS, Perkin–Elmer AAnalyst-800, Waltham, MA) with a flow injection analysis system (Perkin–Elmer FIMS 400) for sample handling. Chromium and Mn were analyzed by graphite furnace atomic absorption spectroscopy (GF-AAS, Perkin–Elmer AAnalyst-800).

2.5. Field-collected plant material

The UC Santa Barbara Riparian Invasives Research Laboratory kindly donated saltcedar foliage from 12 locations in California, Nevada, and Utah. The samples are identified by County, collection date, and GPS coordinates in Table 1. These plants were analyzed as described previously to determine concentrations of perchlorate, Se, Cr, and Mn present in *T. ramosissima* from uncontaminated field sites. These samples provide baseline concentrations of perchlorate, Se, Cr, and Mn in plant samples from California, Nevada, and Utah sites not known to be polluted. These field concentrations also offer a useful contrast with data generated in our laboratory experiments.

2.6. Statistical analysis

Perchlorate and Se larval bioassays were run concurrently, and larval masses were compared using an ANCOVA with starting mass as the covariate, followed by Tukey's HSD as appropriate. Contaminants in water were statistically compared using a repeated-measures ANOVA (Zar, 1996). Concentrations of pollutants in foliage and beetles from the various treatments were log $(x+1)$ transformed (due to both heteroscedasticity and the presence of small numbers) and compared using a *t*-test. All analyses were preformed using SAS statistical software (SAS Institute, 2002) and an alpha of 0.05. All means are reported as ±standard error.

3. Results

3.1. Larval growth

Perchlorate and Se bioassays were carried out simultaneously, and therefore were analyzed together. There was an overall significant effect of contaminant treatment ($F_{2,13} = 12.56$, $P = 0.0009$), with Tukey's post-hoc test revealing that growth of the Se-treated insects (ls mean for larval mass 11.8 ± 1.79 g, $P = 0.0134$), but not the perchlorate-treated insects (ls mean for larval mass 26.1 ± 2.28 g, $P = 0.226$), was significantly different than growth of control insects (ls mean for larval mass 21.0 ± 1.89 g, Fig. 1A). Bioassays of Mn and Cr were each carried out individually at separate time-points, and therefore are analyzed separately. Like perchlorate, neither Mn (ls mean for larval mass: Mn treatment, 6.39 ± 0.739 g, control, 6.24 ± 0.739 g, $F_{1,6} = 0.02$, $P = 0.8911$; Fig. 1B), nor Cr (ls mean for larval mass: Cr treatment, 15.0 ± 1.79 g, control, 17.5 ± 1.84 g, $F_{1,6} = 2.84$, P = 0.143; Fig. 1C) was found to significantly alter larval growth. Of the four contaminants tested, only Se caused a significant reduction (~40% reduction) in larval mass when *D. elongata* larvae fed on treated plants for 7 days. None of the treatments was associated with significant beetle mortality (data not shown).

3.2. Contaminant levels in water

Perchlorate was added to growing water at a nominal level of 100 mg l^{-1}. Over time, the average level in growing water increased, reaching a final level of ~500 mg l^{-1} at the end of the 2-week period (Fig. 2A). Control flasks contained from 0.003 to 0.010 mg l^{-1} of perchlorate. Perchlorate levels were significantly higher in 100 mg l^{-1} perchlorate-treated water across all time-points ($F_{2,12} = 22.55$, $P < 0.001$). There was a significant interaction ($F_{2,12} = 22.55$, $P < 0.001$) between perchlorate treatment and time, indicating that pollutant concentration varied differently through time in the two treatments.

Selenium water levels in the 2 mg l^{-1} Se treatment also increased over time to an average of ~12 mg l^{-1} by the conclusion of the experiment (Fig. 2B). Control flasks with tap water had Se levels from 0.013 to 0.026 mg l^{-1}. Selenium levels were significantly higher in 2 mg l^{-1} Se-treated water than in control treatments ($F_{2,12} = 31.60$, $P < 0.001$). There was a significant interaction between Se treatment and time ($F_{2,12} = 31.43$, $P < 0.001$), indicating that pollutant concentration varied differently through time in the two treatments.

In contrast, Mn levels in 50 mg l^{-1} treatment flasks did not increase over time, and were at all times lower than the nominal treatment level (Fig. 2C), averaging around 10 mg Mn l^{-1}. Levels of Mn in control water averaged 0.001 mg Mn l^{-1}. Manganese levels were significantly higher in 50 mg l^{-1} Mn-treated water ($F_{2,12} = 9.39$, $P = 0.0221$). There was no significant interaction between Mn treatment and time ($F_{2,12} = 0.41$, $P = 0.67$), indicating that the concentration did not vary differently over time in the two treatments.

Lastly, Cr concentrations in 2 mg l^{-1} treatment flasks were somewhat lower than the nominal level (Fig. 2D), averaging 1.4 mg Cr l^{-1}. Levels of Cr in control water averaged around 0.0007 mg Cr l^{-1}. Chromium levels in the 2 mg l^{-1} Cr-treated water were significantly higher-than in control water ($F_{1,6} = 7.84$, $P < 0.001$). There was not a significant interaction between Cr treatment and time ($F_{1,6} = 4.96$, $P = 0.068$), indicating that concentration did not vary differently over time in the two treatments.

3.3. Contaminant levels in plants

Values of contaminants are reported as mg contaminant kg^{-1} dm of plant. An approximate concentration in units of plant fm may be obtained by multiplying the concentration by 0.3 (data not shown). Concentration of perchlorate was significantly greater in plants grown in the 100 mg l^{-1} perchlorate treatment compared to plants grown in control water (perchlorate

Table 1
Foliage concentration of perchlorate, Se, Mn, and Cr from *T. ramosissima* stands in California, Nevada, and Utah.

State	County	Label	GPS	Collection date	Concentrations of pollutants (mg/kg dm)			
					Perchlorate	Selenium	Manganese	Chromium
CA	Kern County	Kern @ I5	35.6181°N, 119.6472°W	5/24/07	<0.0008	0.136	26	1.032
CA	Kern County	Kern @ NW Refuge	35.7205°N, 119.5798°W	5/24/07	<0.0008	<0.136	33.5	0.534
CA	Los Angeles County	Castaic Lake	34.5121°N, 118.6169°W	5/5/07	0.578	<0.136	39	0.340
CA	Riverside County	El Paso #2	33.9060°N, 117.5430°W	5/8/07	0.173	<0.136	74	1.318
CA	Riverside County	El Paso #1	33.9054°N, 117.5395°W	5/8/07	0.021	<0.136	77.5	0.508
CA	San Luis Obispo County	Cuyama River	35.0411°N, 119.8853°W	5/24/07	0.003	<0.136	35.5	0.563
NV	Churchill County	Carson Sink	39.347°N, 118.534°W	8/6/07	0.057	1.809	42.5	0.272
NV	Churchill County	Edwards Creek Basin	39.533°N, 117.715°W	8/6/07	0.040	<0.136	79	0.555
NV	Churchill County	Stillwater Basin	39.439°N, 118.667°W	8/6/07	0.086	0.157	37	0.136
UT	Emery County	Green River	38.99°N, 110.16°W	7/12/07	0.562	<0.136	42	3.686
UT	Grand County	Dewey Bridge	38.8117°N, 109.3026°W	5/7/07	0.014	1.485	58	0.243
UT	Washington County	St George	37.0864°N 113.5556°W	5/8/07	<0.0008	<0.136	25.5	0.338
				Mean	0.171	0.896	47.458	0.794
				SE	0.067	0.253	5.660	0.280

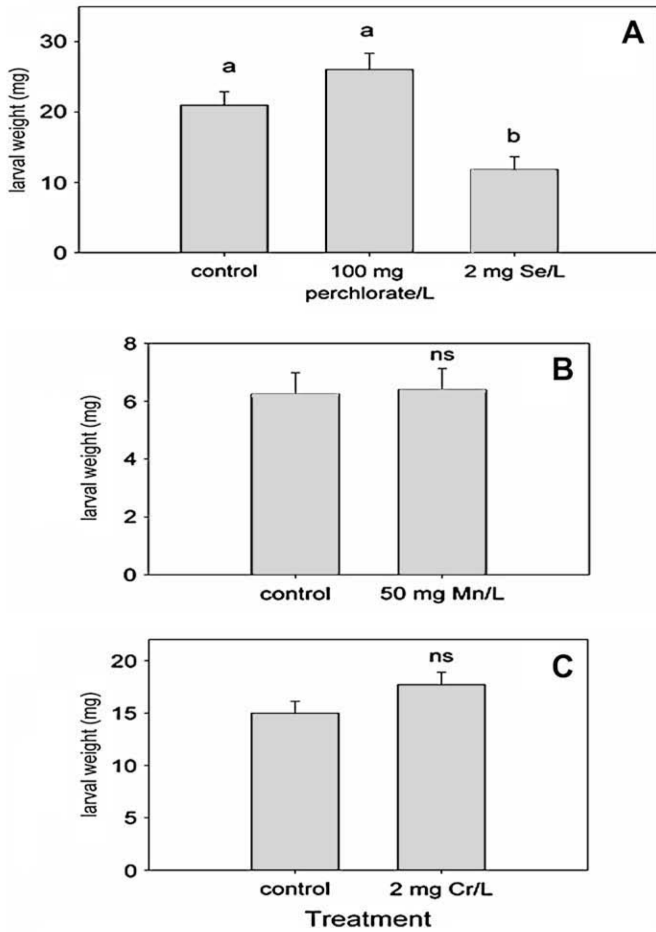

Fig. 1. ls means for final mass of *D. elongata* beetles after feeding for 1 week on *T. ramosissima* plants grown in pollutant-treated or control water. Different letters indicate significant differences.

ls mean 9490 ± 552 mg perchlorate kg^{-1}, control ls mean 59.6 ± 14.6 mg perchlorate kg^{-1}; $F_{1,8} = 616.58$, $P < 0.0001$; Fig. 3A). Selenium accumulated significantly more in plants grown in the 2 mg l^{-1} (ls mean 1070 ± 319 mg kg^{-1}) than in control plants the (ls mean 2.69 ± 0.30 mg Se kg^{-1}, $F_{1,8} = 233.40$, $P < 0.0001$; Fig. 3B). Manganese levels in 50 mg l^{-1} treatment plants had an ls mean of 273 ± 73.2 mg kg^{-1}, vs. 24.0 ± 3.1 mg kg^{-1} in control plants, a significant difference ($F_{1,8} = 54.83$, $P = 0.0003$; Fig. 3C). Finally, plants grown in 2 mg Cr l^{-1} contained an average of 1.89 ± 0.42 mg Cr kg^{-1}, significantly more than the average of 0.04 ± 0.02 mg Cr kg^{-1} in control plants ($F_{1,8} = 34.50$, $P = 0.0011$; Fig. 3D).

3.4. Field-collected plant material

Concentrations of perchlorate, Se, Mn, and Cr from field-collected plants are shown in Table 1. Perchlorate, Se, and Cr concentrations were variable, with levels of perchlorate ranging from <0.0008 to 0.58 mg kg^{-1}, Se from <0.0136 to 1.8 mg kg^{-1}, and Cr from 0.24 to 3.7 mg kg^{-1}. Manganese levels were more uniform, from 26 to 79 mg Mn kg^{-1}.

3.5. Contaminant levels in insect larvae

Concentrations of pollutants in beetles are reported as mg contaminant kg^{-1} (dm). An estimate of concentration per unit of beetle fm may be obtained by multiplying the dm concentration by 0.24 (data not shown). All *D. elongata* feeding on contaminant-treated plants accumulated more contaminant than the respective control insects. Beetles feeding for 1 week on perchlorate-treated plants contained an ls mean of 675 ± 428 mg perchlorate kg^{-1}, vs. 2.27 ± 1.0 mg perchlorate kg^{-1} beetle in the control treatment ($F_{1,8} = 88.75$, $P < 0.0001$; Fig. 4A). Beetles feeding on Se-treated plants accumulated an ls mean of 231 ± 56.9 mg Se kg^{-1}, whereas control beetles accumulated Se at 3.29 ± 0.89 mg Se kg^{-1} ($F_{1,8} = 169.94$, $P < 0.0001$; Fig. 4B). Beetles feeding on Mn-treated plants accumulated an average of 147 ± 87.3 mg Mn kg^{-1}, compared to 15.2 ± 7.48 mg Mn kg^{-1} in control beetles ($F_{1,8} = 21.83$, $P = 0.0034$; Fig. 4C). Finally, beetles feeding on Cr-treated plants

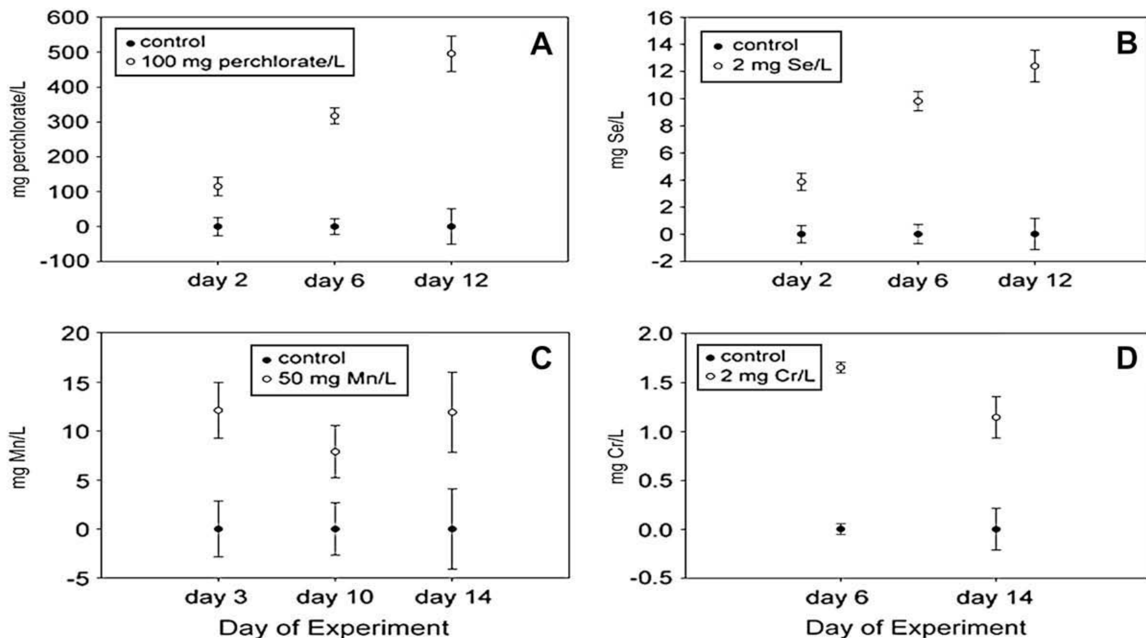

Fig. 2. ls means for concentrations of pollutants in hydroponic growing water over time in pollutant-added and control treatments.

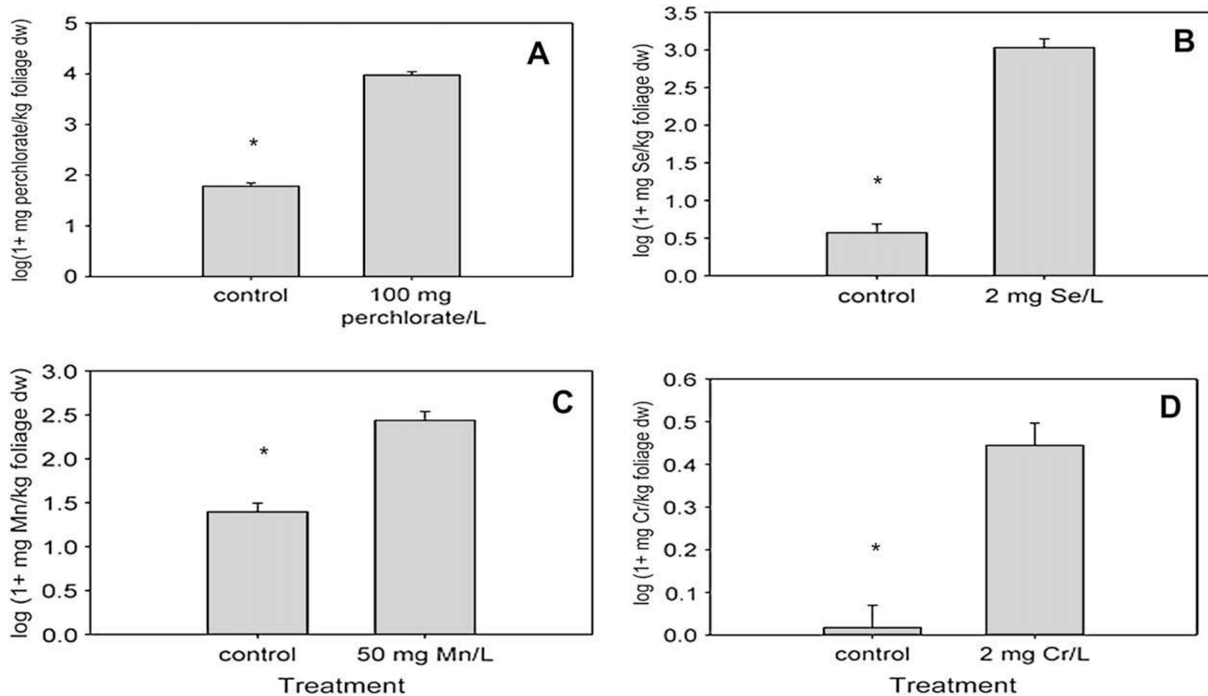

Fig. 3. ls means for log of concentration +1 of pollutants in *T. ramosissima* foliage from plants grown in treatment and control water.

contained 8.42 ± 3.37 mg Cr kg^{-1}, while control beetles contained 3.40 ± 0.71 mg Cr kg^{-1} ($F_{1,8} = 8.05$, $P = 0.0297$; Fig. 4D).

4. Discussion

4.1. Growth of beetle larvae

The observed differences of final larval masses between trials are due to small differences in the age of the larvae used to begin the experiments. Despite significant levels of contaminants in the food plant and in beetle tissue, perchlorate, Mn, and Cr had no effect on the growth of *D. elongata* beetles in the 7-day bioassays at the concentrations tested. Se, however, had a significant effect, decreasing larval growth by about 50%. This decrease in larval growth may, over the course of development, be translated into decreased survival or reproduction, reduced feeding, and overall reduced success where this beetle has been introduced for the biological control of invasive *T. ramosissima*. The selenium treatment used in this study was chosen to reflect the high end of the range of concentrations found in contaminated sites

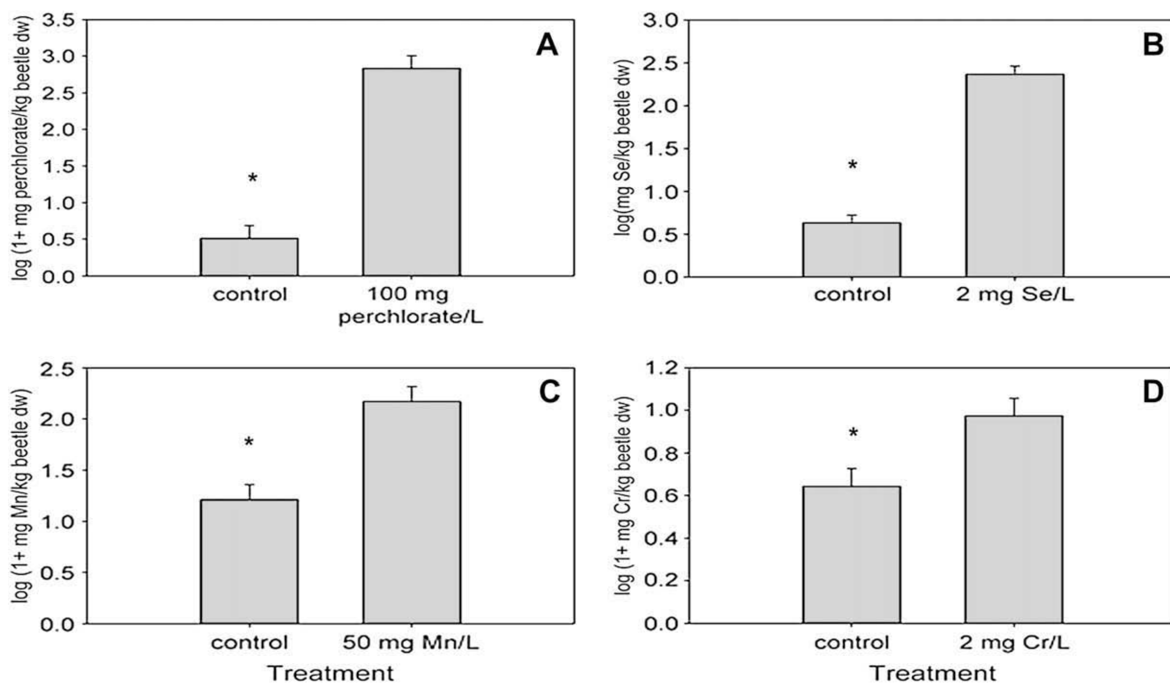

Fig. 4. ls means for log of concentration +1 of pollutants in *D. elongata* beetles after feeding for one week on *T. ramosissima* plants grown in pollutant-treated or control water.

(Seiler et al., 1999). More research will be necessary to determine the threshold Se concentration below which no effect on *D. elongata* fitness is observable. Thus, although *T. ramosissima* clearly has the potential to biaccumulate perchlorate, Se, Mn, and Cr at the concentrations tested, only Se had a demonstrated toxicity to *D. elongata* that may interfere with biological control efforts.

4.2. Contaminant levels in water

Though some perchlorate was taken up by *T. ramosissima*, loss of water through direct evaporation and plant transpiration resulted in a net accumulation of perchlorate in the growing flasks. We speculate that evapoconcentration may also be important in nature where perchlorate-contaminated water is added over time to a closed system such as an aquifer or other isolated surface water. Control flasks contained from 0.003 to 0.012 $mg\,l^{-1}$ perchlorate. Though this is a low level compared to concentrations in treatment flasks, the perchlorate concentration is nonetheless high compared to California's drinking water advisory limit of 0.006 $mg\,l^{-1}$, and further illustrates the potential of evaporation or transpiration to concentrate an extremely stable contaminant like perchlorate.

Selenium concentration in the growing water similarly increased over time, to a maximum of 12 $mg\,l^{-1}$, six times the nominal concentration of 2 $mg\,l^{-1}$. These data illustrate that Se, like perchlorate, becomes concentrated through evaporation or transpiration of water. Evapoconcentration is thought to be responsible for elevated levels of Se in large areas of Central California (Seiler et al., 1999).

Though Mn was added to the hydroponic growing water at 50 $mg\,Mn\,l^{-1}$, analysis of water samples found only 8–12 $mg\,Mn\,l^{-1}$. The solution was not saturated with dissolved Mn, as $MnCl_2$ has a solubility of 723 $g\,l^{-1}$. Additionally, the abiotic oxidation of soluble Mn (II) to insoluble Mn (IV) is very slow (Morgan, 2000). Manganese oxidizing bacteria, however, are known to precipitate Mn in aquatic systems (Chapnick et al., 1982; Johnson et al., 1995). The bacteria often cling to hard surfaces, creating a biofilm that turns brownish-black due to deposits of insoluble Mn (VI) (King et al., 1999). Observations during our study were consistent with the presence of Mn-oxidizing bacteria in our hydroponic growing flasks. While control, perchlorate, Se, and Cr treatments contained visible green algae, our Mn treatment flasks contained a dark brown coating, and little or no green algae, consistent with another study noting the replacement of green algae with a brown bacterial biofilm (King et al., 1999). Although insoluble Mn (IV) was likely present in treatment flasks, only soluble Mn (II) is bioavailable to plants (Morgan, 2000), so the measured water concentration should be considered the actual amount of Mn the plants were exposed to. Clearly, elevated levels of Mn were bioavailable to treatment plants, as evidenced by the higher Mn content of foliage from treatment than from control plants.

Levels of Cr in water maintained a level slightly below the 2 $mg\,l^{-1}$ nominal treatment level. Though Cr (VI) is soluble in water at most pH values, dissolved Cr (VI) is precipitated by divalent cations, of which iron (Fe) and dissolved organic matter are considered to be the most important (Stanin, 2005). In our treatment flasks, some Cr (VI) was likely precipitated out of solution due to cations or organic matter such as the algae observed to be present in the most of the treatment solutions.

The observed increases and decreases in contaminant levels in water are not surprising due to the complexity of the chemical interactions involved. In the field we predict that plants in a riparian environment may often experience fluctuating exposure to contaminants. Herbivorous insects, however, will are likely to experience more constant exposure as plants gradually acquire or excrete contaminants. In this study, the changes in contaminant concentration.

4.3. Contaminant levels in plants

T. ramosissima used in this study were grown from cuttings, with foliage ≤6 weeks old. Additionally, care was taken to ensure that neither new nor senescent foliage was used for bioassays and for chemical analysis. In the field, contaminant concentration will likely vary will foliage age, and the effect this variation may have on *D. elongata* is not known. Additionally, this experiment was conducted over a relatively short time frame, and there were fluctuations in contaminant concentrations in treatment water. However, we speculate that pollution accumulation, and likely effects, would be even greater for plants exposed for longer periods and the results that we present should be considered conservative. Overall, we observed that *T. ramosissima* readily accumulated pollutants in its foliage, though the extent of the accumulation varied according to the particular contaminant.

Perchlorate levels in the 12 field-collected plant samples were much lower than perchlorate levels in control plants from our hydroponic greenhouse studies (mean 0.17 $mg\,kg^{-1}$ in field plants vs. mean 60 mg perchlorate kg^{-1} dm in hydroponic controls). Elevated levels of perchlorate in our hydroponic controls may have been due to contamination in the original cuttings from which the plants were grown, or from the commercial nutrient solution used to grow the plants. Alternatively, perchlorate levels may be lower in field plants because they were collected from areas with perchlorate levels lower than those in our control treatment water (<0.003–0.010 $mg\,l^{-1}$). Perchlorate-treated plants accumulated a surprising 10,000 mg perchlorate kg^{-1}.

At an average of 1250 $mg\,Se\,kg^{-1}$ dm, Se-treated *T. ramosissima* had as much Se as hyperaccumulator plants (plants that accumulate >1000 $mg\,Se\,kg^{-1}$ fm; Reeves and Baker, 2000).

According to a review by Terry et al. (2000), crop plants grown on non-seleniferous soil typically contain from 0.05 to 0.10 $mg\,Se\,kg^{-1}$ dm, whereas non-hyperaccumulator plants grown on seleniferous soil rarely accumulate more than 100 $mg\,Se\,kg^{-1}$ dm. Our field-collected *T. ramosissima* plants averaged 0.9 $mg\,Se\,kg^{-1}$, somewhat more than the above values given for crop plants on non-seleniferous soil, but lower than the average of 3 $mg\,Se\,kg^{-1}$ in our hydroponic control plants. As noted for perchlorate, field-collected plants may have been exposed to less Se than our control plants (<0.013–0.026 $mg\,Se\,l^{-1}$), or residual Se from cuttings or nutrient solution may have been present.

Field plants had a concentration of Mn higher-than our hydroponic controls (47 vs. 24 $mg\,Mn\,kg^{-1}$, respectively). Treated plants had higher levels of Mn than field-collected plants, averaging 270 $mg\,Mn\,kg^{-1}$. Healthy plants have been reported to contain a wide range of levels of Mn, with values ranging from 5 to 1500 $mg\,Mn\,kg^{-1}$ (Raven et al., 1976). Salisbury and Ross (1978) report 50 $mg\,Mn\,kg^{-1}$ to be a more "typical" value for levels of Mn in plants. Thus, although all plant samples had levels of Mn within the "normal" range, field plants were at a "typical" level, with control plants being somewhat below the typical value, and Mn-treated plants having a higher-than typical level of Mn (270 $mg\,kg^{-1}$).

Control plants contained 0.04 $mg\,Cr\,kg^{-1}$, compared to a much higher average of 0.8 $mg\,Cr\,kg^{-1}$ in field plants. Interestingly, plants from one location, the "Green River" site in Emory County, UT, had a concentration of Cr higher even than our 2 $mg\,Cr\,l^{-1}$ treatment plants (3.69 vs. 1.90 $mg\,Cr\,kg^{-1}$, respectively). This site likely has higher-than-average levels of soil Cr, either naturally or due to anthropogenic contamination. A review by Pawlisz et al. (1997) reported that plants from unpolluted, non-ultramafic soils usually contain less than 1 $mg\,Cr\,kg^{-1}$.

4.4. Contaminant levels in insect larvae

Both treatment and control insects (1600 and 3 mg perchlorate kg^{-1}, respectively) accumulated less perchlorate than the plants (perchlorate-treated: 10,000 mg kg^{-1}, control: 60 mg kg^{-1}), indicating that insects were able to excrete some perchlorate. This finding agrees with a report from Smith et al. (2001), where damselflies exposed to 31 mg l^{-1} perchlorate in water contained only 8–20 mg perchlorate kg^{-1} insect fm. Nonetheless, perchlorate in insects may become bioavailable to more perchlorate-sensitive taxa, such as insectivorous mammals.

Levels of Se in insects from both our control (4 mg kg^{-1}) and Se treatments (260 mg kg^{-1}) contained more Se than control insects in a study by Vickerman and Trumble (2003), where larval *Spodoptera exigua* contained less than 1 mg Se kg^{-1}. In the same study, insects feeding on diet treated with 109 or 135 mg Se kg^{-1} accumulated 10 or 13 mg Se kg^{-1} insect. These results are somewhat comparable to ours, where insects feeding on foliage containing 1250 mg Se kg^{-1} were able to excrete much of the Se they were exposed to, ending up with a final concentration of 260 mg Se kg^{-1}.

In contrast to *D. elongata*'s excretion of perchlorate and Se, Mn-treated insects accumulated only slightly less Mn (200 mg kg^{-1}) than their food plants (270 mg Mn kg^{-1}), while control insects contained approximately the same concentration of Mn (23 mg Mn kg^{-1}) as control plant foliage (24 mg Mn kg^{-1}). Levels of Mn were much higher in both treatment and control insects than levels reported by other authors. Davison et al. (1999) found that insects from non-ultramafic sites in Scotland contained <5 mg Mn kg^{-1}, while only one order of insect, the Hemiptera, contained significantly more Mn (20 mg Mn kg^{-1}) when it was collected from ultramafic sites.

Levels of Cr were elevated in both beetles feeding on both Cr-treated plants (12 mg Cr kg^{-1} beetle) and beetles feeding on control plants (4 mg Cr kg^{-1} beetle), compared to a study by Popham and Shelby (2006), where larval *Heliothis virescens* contained 0.002–0.02 mg Cr kg^{-1} after feeding on a "control" artificial diet containing 0.39 mg Cr kg^{-1}. Davison et al. (1999) found comparable levels (<0.05 mg Cr kg^{-1}) in insects from non-ultramafic sites in Scotland, while insects in one order, Hemiptera, when collected from ultramafic sites contained as much as 0.5 mg Cr kg^{-1}. Although the presence of Cr in food plants was not found to harm *D. elongata* beetles themselves, Cr may become available through these insects to other parts of the food chain, such as insectivorous birds.

The bioassays in this study contained a low number (four) of plant replicates. However, variance was nonetheless low enough, and treatment effects large enough, for statistical conclusions to be drawn from the data. Additionally, although this study involved chronic, 1-week bioassays, in the field insects would likely be exposed to pollutants over their entire life-cycle, possibly leading to toxicity at lower pollutant concentrations and/or developmental or reproductive effects not measured by this study.

It should be noted that even if these contaminants do not harm *D. elongata*, the pollutants may have consequences for other herbivore species feeding on *T. ramosissima*, or even other trophic levels. Although this beetle was introduced into the United States without its normal complement of parasitoids and predators, native parasitoids and predators may host-shift to take advantage of the new resource. Ultimately, these insects, and the detritivores that feed on the additional resource of dead plant material, would be exposed to elevated concentrations of these metals and metalloids. While some parasitoids and predators have the ability to minimize their intake of these contaminants, there are nonetheless substantial impacts on fitness correlates such as weight or adult longevity (Vickerman and Trumble, 2003; Butler and Trumble, in press). Nonetheless, susceptibility of parasitoids, predators, and detritivores will depend on the species in question, and extended ecological effects remain difficult to predict.

Because the plant grows in disturbed areas, acquires salts readily, and utilizes groundwater, *T. ramosissima* seems a likely candidate for exposure to and acquisition of a variety of anthropogenic pollutants. Thus, it is perhaps not surprising that the specialist herbivore *D. elongata* was tolerant of the contaminants tested here. We found that although *T. ramosissima* bioaccumulated perchlorate, Se, Mn, and Cr, and these contaminants were transferred to the biological control agent *D. elongata*, perchlorate, Mn, and Cr did not affect *D. elongata* development. Selenium, however, significantly reduced larval growth over a 1-week bioassay period. This study is one of the first to demonstrate that a contaminant taken up by a weed can have effects on an insect herbivore biological control agent.

Acknowledgements

R.I. Carruthers and J. Herr of the USDA Invasive Weeds research unit in Albany, CA, provided eggs and rearing advice for the establishment of our *D. elongata* colony. A. Seyfferth assisted with perchlorate analysis, and W. Smith and K. Thrippleton provided help with metal analysis. Thank you to Tom Dudley and the UCSB Riparian Invasives Research Lab for donating field-collected saltcedar foliage. K. Gilbert, C.M. Chase-Dunn, B. Gonzalez, W. Carson, and G. Kund helped with various aspects of the project. Earlier drafts of this manuscript were improved by the comments of R. Redak and W. Walton.

References

Arndt, S.K., Arampatsis, C., Foetzki, A., Li, X.Y., Zeng, F.J., Zhang, X.M., 2004. Contrasting patterns of leaf solute accumulation and salt adaptation in four phreatophytic desert plants in a hyperarid desert with saline groundwater. Journal of Arid Environments 59, 259–270.

ATSDR (Agency for Toxic Substances and Disease Registry), 1997. Toxicological Profile for Manganese (Update). Draft for Public Comment. U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.

Berry, W.L., 1970. Characteristics of salts secreted by *Tamarix aphylla*. American Journal of Botany 53, 1226–1230.

Butler, C.D., Trumble, J.T., 2008. Effects of pollutants on bottom-up and top-down processes in insect-plant interactions. Environmental Pollution 156, 1–10.

Brown Jr., G.E., Foster, A.L., Ostergren, J.D., 1999. Mineral surfaces and bioavailability of heavy metals: a molecular-scale perspective. Proceedings of the National Academy of Sciences 96, 3388–3395.

Chapnick, S.D., Moore, W.S., Nealson, K.H., 1982. Microbially mediated manganese oxidation in a freshwater lake. Limnology and Oceanography 27, 1004–1014.

Coleman, C.M., Boyd, R.S., Eubanks, M.D., 2005. Extending the elemental defense hypothesis: dietary metal concentrations below hyperaccumulator levels could harm herbivores. Journal of Chemical Ecology 31, 1669–1681.

Davison, G., Lambie, C.L., James, W.M., Skene, M.E., Skene, K.R., 1999. Metal content in insects associated with ultramafic and non-ultramafic sites in the Scottish Highlands. Ecological Entomology 24, 396–401.

DeLoach, C.J., Lewis, P.A., Herr, J.C., Carruthers, R.I., Tracy, J.L., Johnson, J., 2003. Host specificity of the leaf beetle, *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae) from Asia, a biological control agent for saltcedars (*Tamarix*: Tamaricaceae) in the western United States. Biological Control 27, 117–147.

DeLoach, C.J., Carruthers, R., Dudley, T., Eberts, D., Kazmer, D., Knutson, A., Bean, D., Knight, J., Lewis, P., Tracy, J., Herr, J., Abbot, G., Prestwich, S., Adams, G., Mityaev, I., Jashenko, R., Li, B., Sobhian, R., Kirk, A., Robbins, T., Delfosse, E., 2004. First results for control of saltcedar (*Tamarix* spp.) in the open field in the western United States. In: Cullen, J. (Ed.), Proceedings, XI International Symposium on the Biological Control of Weeds, April 27-May 3, 2003, Canberra, Australia.

Gate, I.M., McNeill, S., Ashmore, M.R., 1995. Effects of air pollution on the searching behaviour of an insect parasitoid. Water, Air, and Soil Pollution 85, 1425–1430.

Holton, K.M., Lindroth, R.L., Nordheim, E.V., 2003. Foliar quality influences tree–herbivore–parasitoid interactions: effects of elevated CO_2, O_3, and plant genotype. Oecologia 137, 233–244.

Howe, P.D., Malcolm, H.M., Dobson, S., 2004. Manganese and its Compounds: Environmental Aspects. Concise International Chemical Assessment Document: 63. World Health Organization, Geneva, Switzerland.

Jensen, P., Arias, M., Trumble, J.T., 2005. Toxicological and developmental responses of a terrestrial insect detritivore, *Megaselia scalaris* (Leow) to four selenium species. Ecotoxicology 14, 311–320.

Jensen, P.D., Johnson, L.R., Trumble, J.T., 2006. Individual and joint actions of selenate and methylmercury on the development and survival of insect detritivore *Megaselia scalaris* (Diptera: Phoridae). Archives of Environmental Contamination and Toxicology 50, 523–530.

Jensen, P.D., Sorensen, M.A., Walton, W.E., Trumble, J.T., 2007. Lethal and sublethal responses of an aquatic insect *Culex quinquefasciatus* (Diptera: Culicidae) challenged with individual and joint exposure to dissolved sodium selenate and methyl mercury chloride. Environmental Toxicology 22, 287–294.

Johnson, D., Chiswell, B., O'halloran, K., 1995. Micro-organisms and manganese cycling in a seasonally stratified freshwater dam. Water Research 29, 2739–2745.

Kazimirova, M., Slovak, M., Manova, A., 1997. Host–parasitoid relationship of *Ceratitis capitata* (Diptera: Tephritidae) and *Coptera occidentalis* (Hymenoptera: Proctotrupoidea: Diapriidae) under host heavy metal stress. European Journal of Entomology 94, 409–420.

King, H.M., Baldwin, D.S., Rees, G.N., McDonald, S., 1999. Apparent bioaccumulation of Mn derived from paper-mill effluent by the freshwater crayfish Cherax destructor – the role of Mn oxidizing bacteria. Science of the Total Environment 226, 261–267.

Leslie, H.A., Pavluk, T.I., bij de Vaate, A., Kraak, M.H.S., 1999. Triad assessment of the impact of chromium contamination on benthic macroinvertebrates in the Chusovaya River (Urals, Russia). Archives of Environmental Contamination and Toxicology 37, 182–189.

Los Angeles Regional Water Quality Control Board, 2004. Available from: <http://www.swrcb.ca.gov/rwqcb4/html/water_qty/chromium_S1.html>.

Martin-Romero, F.J., Kryukov, G.V., Lobanov, A.V., Carlson, B.A., Lee, B.J., Gladyshev, V.N., Hatfield, D.L., 2001. Se metabolism in *Drosophila*: selenoproteins, selenoprotein mRNA expression, fertility and mortality. Journal of Biological Chemistry 276, 29798–29804.

Milward, C.G., Kuckner, P.D., 1989. Microwave digestion technique for the extraction of minerals from environmental marine sediments for analysis by inductively coupled plasma atomic emission spectrometry and atomic absorption spectrometry. Journal of Analytical Atomic Spectrometry 4, 709–713.

Morgan, J.J., 2000. Manganse in natural waters and earth's crust: its availability to organisms. In: Sigel, A., Sigel, H. (Eds.), Metal Ions in Biological Systems. Manganese and its Role in Biological Processes, vol. 37. Marcel Dekker, NY, New York, pp. 1–30.

Pawlisz, A.V., Kent, R.A., Schneider, U.A., Jefferson, C., 1997. Canadian water quality guidelines for chromium. Environmental Toxicology and Water Quality 12, 123–183.

Popham, H.J.R., Shelby, K.S., 2006. Uptake of dietary micronutients from artificial diets by larval *Heliothis virescens*. Journal of Insect Physiology 52, 771–777.

Raven, P.H., Evert, R.F., Curtis, H., 1976. Biology of Plants, second ed. Worth, New York, NY.

Reeves, R.D., Baker, A.J., 2000. Metal accumulating plants. In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of Toxic Metals: Using Plant to Clean up the Environment. John Wiley and Sons, New York, NY, USA, pp. 193–229.

Salisbury, F.B., Ross, C.W., 1978. Plant Physiology, second ed. Wadsworth, Belmont, CA.

SAS Institute, 2002. SAS Statistical Software. SAS Institute, Cary, New York, NY.

Seiler, R.L., Skorupa, J.P., Peltz, L.A., 1999. Areas susceptible to irrigation-induced selenium contamination of water and biota in the western United States. USGS Survey: 1180.

Seyfferth, A.L., Parker, D.R., 2006. Determination of low levels of perchlorate in lettuce and spinach using ion chromatography–electrospray ionization mass spectrometry (IC–ESI–MS). Journal of Agricultural and Food Chemistry 54, 2012–2017.

Shafroth, P.B., Cleverly, J.R., Dudley, T.L., Taylor, J.P., Van Riper, C., Weeks, E.P., Stuart, J.N., 2005. Control of *Tamarix* in the Western United States: implications for water salvage, wildlife use, and riparian restoration. Environmental Management 35, 231–246.

Smith, P.N., Theodoraakis, C.W., Anderson, T.A., Kendall, R.J., 2001. Preliminary assessment of perchlorate in ecological receptors at the Longhorn Army Ammunition Plant (LHAAP), Karnack, Texas. Ecotoxicology 10, 305–313.

Sorensen, M.A., Jensen, P.D., Walton, W.E., Trumble, J.T., 2006. Acute and chronic activity of perchlorate and hexavalent chromium contamination on the survival and development of *Culex quinquefasciatus* Say (Diptera: Culicidae). Environmental Pollution 144, 759–764.

Stanin, F.T., 2005. The transport and fate of chromium (VI) in the environment. In: Guertin, J., Jacobs, J.A., Avakian, C.P. (Eds.), Chromium (VI) Handbook. CRC, Boca Raton, FL, pp. 165–199.

Storey, R., Thomson, W.W., 1994. An x-ray microanalysis study of the salt glands and intracellular calcium crystals of *Tamarix*. Annals of Botany 73, 307–313.

Terry, N., Zayed, A.M., de Souza, M.P., Tarun, A.S., 2000. Selenium in higher plants. Annual Review of Plant Physiology and Plant Molecular Biology 51, 401–432.

Thomson, W.W., Berry, W.L., Liu, L.L., 1969. Localization and secretion of salt by the salt glands of *Tamarix aphylla*. Proceedings of the National Academy of Sciences 63, 310–317.

Trager, W., 1953. Insect nutrition. In: Roder, K.D. (Ed.), Insect Physiology. Chapman and Hall, London.

Trumble, J.T., Jensen, P.D., 2004. Ovipositional response, developmental effects and toxicity of hexavalent chromium to *Megaselia scalaris*, a terrestrial detritivore. Archives of Environmental Contamination and Toxicology 46, 372–376.

Urbansky, E.T., Magnuson, M.L., Kelty, C.A., Brown, S.K., 2000. Perchlorate uptake by salt cedar (*Tamarix ramosissima*) in the Las Vegas Wash riparian ecosystem. The Science of the Total Environment 256, 227–232.

US EPA, 2004. Known perchlorate releases in the US. Available from: <http://www.epa.gov/swerffrr/documents/perchlorate_map/nationalmap.htm>.

Vajpayee, P., Sharma, S.C., Tripathi, R.D., Rai, U.N., Yunus, M., 1999. Bioaccumulation of chromium and toxicity to photosynthetic pigments, nitrate reductase activity and protein content of *Nelumbo nucifera* Gaertn. Chemosphere 39, 2159–2169.

Vickerman, D.B., Young, J., Trumble, J.T., 2002. Effect of selenium-treated alfalfa on development, survival, feeding and oviposition preferences of *Spodoptera exigua* (Lepidoptera: Noctuidae). Environmental Entomology 31, 953–959.

Vickerman, D.B., Trumble, J.T., 2003. Biotransfer of selenium: effects on an insect predator, *Podisus maculiventris*. Ecotoxicology 12, 497–504.

Voorhees, M.E., Uresk, D.W., 1990. Evaluation of rillscale for herbivore forage on bentonite mine spoils. Northwest Science 64, 181–186.

White, P.J., Bowen, H.C., Marshall, B., Broadley, M.R., 2007. Extraordinarily high leaf selenium to sulfur ratios define 'Se-accumulator' plants. Annals of Botany 100, 111–118.

World Bank, 2002. Available from: <http://www.worldbank.org/nipr/polmod.htm>.

Zar, J.H., 1996. Biostatistical Analysis, third ed. Prentice-Hall Inc., New Jersey, USA.

Zayed, A., Lytle, C.M., Qian, J.H., Terry, N., 1998. Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta 206, 293–299.

Zayed, A.M., Terry, N., 2003. Chromium in the environment: factors affecting biological remediation. Plant and Soil 249, 139–156.