Science of the Total Environment 481 (2014) 446–452

Contents lists available at ScienceDirect

Science of the Total Environment

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

Impact of selenium on mortality, bioaccumulation and feeding deterrence in the invasive Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae)

Deborah G. De La Riva [a,*], Beatriz G. Vindiola [a], Tracy N. Castañeda [a], David R. Parker [b], John T. Trumble [a]

[a] Department of Entomology, University of California, Riverside, 900 University Ave., Riverside, CA 92521, USA
[b] Department of Environmental Sciences, University of California, Riverside, 900 University Ave., Riverside, CA 92521, USA

HIGHLIGHTS

- The toxicity of Se compounds for invasive Argentine ants was tested.
- Methylselenocysteine was the most toxic of the four chemical forms tested.
- Bioaccumulation and toxicity were dependent on form and concentration.
- Selenium did not act as a deterrent to ant feeding in choice test assays.
- Selenium was not repellant, regardless of the background sucrose concentration.

ARTICLE INFO

Article history:
Received 26 November 2013
Received in revised form 6 February 2014
Accepted 8 February 2014
Available online 12 March 2014

Keywords:
Methylselenocysteine
Pollution
Selenate
Selenite
Selenomethionine

ABSTRACT

Ants are known for the important roles they play in processes contributing to ecosystem functioning in many habitats. However, pollutants can impact the ecosystem services provided by ants. The Argentine ant, an invasive species in North America, was investigated for the potential impact selenium (Se) may have on ants residing within a contaminated habitat. Mortality tests were conducted using worker ants fed an artificial nectar source containing 1-of-4 environmentally common Se compounds (forms): seleno-L-methionine, methylselenocysteine, selenate or selenite. Accumulation of Se in ant bodies at the end of two weeks was quantified with the use of hydride generation atomic absorption spectroscopy. Lastly, we conducted choice tests using dyes to determine whether ants might avoid a carbohydrate diet containing Se by providing them a choice between sucrose with or without Se. Choice tests also tested the responses of ants to selenium when provided in different background sucrose concentrations. The results of this study indicated that form and quantity of Se, as well as time of exposure, impact mortality in Argentine ant workers. Methylselenocysteine and selenate were found to be the most toxic among the 4 chemical forms when presented in sucrose solutions, whereas seleno-L-methionine and selenite caused greater Se body burdens. Furthermore, choice tests showed that ants did not prefer control sucrose solution to sucrose treated with Se regardless of the background sucrose concentration. These findings serve as first look into the possible detrimental impacts these contaminants may pose for ants that frequent sugary nectar sources.

1. Introduction

Ecosystem services provided by insects in the United States are estimated at a value of $50 million yr^{-1} (Losey and Vaughan, 2006). Ants are considered to be keystone species and 'ecosystem engineers' in many ecological communities (Power et al., 1996; Folgarait, 1998; Lach et al., 2010) due to the critical roles and ecosystem services they provide. These fundamental processes include nutrient cycling (Petal et al., 1977; MacMahon et al., 2000), soil aeration (Folgarait, 1998), pollination (Gómez et al., 1996), seed dispersal (Samson et al., 1992; MacMahon et al., 2000; Christian, 2001), and natural pest control (Perfecto, 1991; Vandermeer et al., 2002; Rosumek et al., 2009).

Natural and anthropogenic disturbances that impact ants may have adverse implications for ecosystem functioning (Vanbergen and Initiative, 2013). Previous studies have reported negative impacts of heavy metal pollution from smelters on wood ant physiology (Sorvari et al., 2007), nest mound volumes and abundance (Eeva et al., 2004),

* Corresponding author. Tel./fax: +1 951 827 4297.
E-mail address: ddela005@ucr.edu (D.G. De La Riva).

http://dx.doi.org/10.1016/j.scitotenv.2014.02.060

and on abundance and richness in ant populations (Hoffmann et al., 2000). Not all trends were negative (Grześ, 2009), because ant species have considerable variation in metal regulation physiology (Grześ, 2010). However, the literature does lack evidence for the impacts of other lesser-known but common contaminants on ants, such as the metalloid selenium.

Selenium (Se) is a widespread and naturally occurring element. Within the U.S. Se is naturally abundant in the Rocky Mountain and Central Plains, as well as areas in the Pacific Northwest and Southwest (Eisler, 2007). Due to its abundance in the western United States (Brown et al., 1999), approximately 414,400 km^2 of land is susceptible to rainfall or irrigation-induced Se contamination (Seiler et al., 1999). It also exists in the environment in different oxidation states (Hoffmann, 2003) occurring in both inorganic and organic forms depending on soil conditions and biological activity in plants or animals (Mayland, 1994; Daniels, 1996; Eisler, 2007). Soluble selenates and selenites are species most readily available to plants (Hoffmann, 2003). These can be found in the inorganic form within the plant or be converted to organics such as selenomethionine (SeMet), methylselenocysteine (MeSeCys), and selenocysteine (Hoffmann, 2003; Eisler, 2007; Bañuelos et al., 2011; Quinn et al., 2011).

Plant species adapted for survival in seleniferous soils are often capable of sequestering higher concentrations of Se in their tissue relative to that in the soil (Eisler, 2007; Babula et al., 2008). Plant components consumed by herbivorous/granivorous species of ants such as the seeds, pollen, and nectar (Rico-Gray and Oliveira, 2007; Lach et al., 2010) have been discovered to bioconcentrate high concentrations of Se. For instance, nectar was reported to contain over 100 μg Se ml^{-1} (fresh weight), and pollen contained over 1000 μg Se g^{-1} (dry weight) in selenium accumulating plants within the family Brassicaceae (Hladun et al., 2011; Quinn et al., 2011). Another study found that the prickly pear cactus, *Opuntia ficus-indica*, had the ability to concentrate approximately 17 μg Se g^{-1} in the seeds, 47 μg Se g^{-1} in the fruit, and over 100 μg Se g^{-1}in cladodes (all dry weight) (Bañuelos et al., 2011). Selenium is essential for growth in both humans and animals (Council, 1983; Daniels, 1996) yet, can also lead to toxicity if nonspecifically incorporated into proteins and enzymes in place of sulfur (Daniels, 1996). Accumulating plants have been considered for their potential to phytoremediate contaminated sites (Parker and Page, 1994), but if Se concentrations accumulated by tolerant plants exceed dietary requirements, they may also pose risks for wildlife ingesting these plants (Eisler, 1985).

The majority of studies investigating Se impact on wildlife have been conducted on mammals, fish, and birds, with comparatively few studies on invertebrates (Ohlendorf and Santolo, 1994; Vickerman et al., 2002; Vickerman and Trumble, 2003; Jensen et al., 2005; Hladun et al., 2012). There is no published study to date investigating the effects of environmental Se on ant communities, despite their abundance and close association with plants (Folgarait, 1998; Rico-Gray and Oliveira, 2007). The ant–plant relationship provides a novel system for studying the toxic effects of Se, because ant feeding behavior allows for acquisition of toxins that may be present in the nectar, extrafloral nectaries, seeds, pollen, as well as herbivore prey, and honeydew. Honeydew, a sugary substance secreted by many phloem-feeding insects (Styrsky and Eubanks, 2007), has been found to be a source of heavy metal transfer to ants (Migula and Głowacka, 1996). A study by Markin (1970a) in southern California citrus groves reported 99% of food brought back to the nest by foragers of the invasive Argentine ant, *Linepithema humile*, consisted of honeydew. Well known for this sugar-seeking behavior and this species' occurrence in the southwestern US (Klotz et al., 2008), the Argentine ant was chosen as an initial model organism for investigation into the impact of selenium on invertebrates.

We hypothesized that Se concentrations in the environment available to foraging ants would be detrimental. However, we were unaware as to the degree of toxicity posed by various selenocompounds and whether or not ants would indeed be willing to ingest these compounds. This study was then conducted with the following objectives in mind: 1) identify a difference, if any, in toxicity between various selenocompounds (forms) 2) determine whether Se is accumulated in ants, and 3) determine if Se might act as a repellent to ant feeding.

2. Materials and methods

2.1. Ant collections and rearing

Argentine ant colonies containing queens, brood and workers were collected in October 2011, June and July of 2012, and February 2013 from the Department of Agricultural Operations at the University of California, Riverside. Colonies were then transferred into plastic containers (31 × 26 × 10 cm) where inner walls had been coated with liquid Teflon (DuPont™ Teflon® PTFE TE-3859) to prevent escape. Nesting material was provided in the form of 9 cm diameter plastic Petri dish bottoms filled with DAP® Plaster of Paris (dry mix) and covered with cardboard to provide darkness. Nests were moistened with de-ionized water twice a week to maintain humidity. Water was provided in a cotton-plugged 50-ml falcon tube along with weekly replenishments of chopped cockroaches, *Periplaneta americana* (as a protein source), and 25% sucrose water (3 times per week). Rearing was conducted under ambient laboratory conditions of 24 ± 1 °C, 40 ± 10% RH and LD: 14:10. Colonies were allowed to acclimate to laboratory conditions for approximately 4–5 days before use in experiments. Only worker ants were used for all experiments.

2.2. Mortality assays

Assays were carried out for 2 weeks in order to investigate the effects of chronic ingestion of four selenocompounds (forms) purchased from Sigma Aldrich, St. Louis, MO; selenate as sodium selenate (Na_2SeO_4), selenite as sodium selenite (Na_2SeO_3), MeSeCys, and SeMet. In order to determine the LC_{50} (lethal concentration that kills 50% of the population) a range of concentrations was tested for each form. Chemicals were incorporated into solutions of 25% sucrose to achieve concentrations of 0, 2, 4, 10, 20, 30, 40 and 50 μg Se ml^{-1} for all forms but selenate for which concentrations were 0, 0.5, 2.7, 5.4, 13.5, 27, and 54 μg Se ml^{-1} (concentrations were different for selenate due to an initial calculation error, but still provided a sufficient range with which to calculate an LC_{50}). Each replicate consisted of a plastic box (30 × 18 × 10.5 cm^3) containing 100 worker ants, water (cotton-plugged 50-ml falcon tube), and shelter. Shelter was similar to that provided for colony rearing except only 5 cm diameter Petri dishes were used. Ants were allowed to acclimate overnight, and dead ants (injured from transport) were removed the next day, and replaced with live ants. Following the acclimation period, a 10 ml vial containing one of the test concentrations for a particular Se form was then added to each box. At least 3 replicates were conducted for each form. Worker ants were allowed to feed *ad libitum*. The number of dead ants was recorded, and they were removed from each box every 24 ± 2 h following initial feeding. Cotton for water and treatment vials was checked daily to ensure that it remained moist. In addition, the treatment vials were replaced with new ones at the end of one week to prevent interference from microbial growth in the sucrose solutions.

2.3. Analysis of Se accumulation

In order to quantify concentrations that may occur within ant populations foraging in contaminated habitats, we chose to analyze the surviving ants from each mortality assay. Because single ants did not provide enough material for Se analysis, all ants from a single box were combined and sacrificed by freezing. Ants were stored in a freezer (−60 °C) prior to freeze-drying (Labconco Corp., Kansas City, MO) at −40 °C at −25 psi for 48 h. Dry weights were then measured using a microbalance before microwave digestion.

Ant tissue was microwave digested in 110 ml Teflon-lined vessels containing 5 ml HNO_3 at 200 °C for 30 min (CEM Corp., Matthews, NC). Digested filtrate was then gently heated on a hot plate for approximately 10 min to remove any excess NO_x gases. The resulting filtrate was diluted in 6 M HCl and heated in a water bath at 90 °C for 20 min prior to analysis for accumulated Se using hydride generation atomic absorption spectroscopy (HG-AAS; Perkin-Elmer, Waltham, MA). Quality control was verified with NIST Standard Reference Material (oyster, NIST 1566B), with an average percent recovery >90%.

2.4. Deterrence (choice-test) assays

We investigated whether or not foraging ants would exhibit a preference between a diet containing Se and a diet without Se. For this, arenas were constructed (Supplemental Fig. 1) that consisted of 4 feeding stations, 2 untreated controls, and 2 treatment stations containing 50 μg Se ml^{-1} of each form of Se placed in an alternating fashion in the bottom center of a plastic box (30 cm × 18 cm × 10.5 cm (height)). As part of this study, control and treatment solutions were also prepared in 10% and 30% sucrose solutions to determine if differences in concentration of sugar in nectar are likely to influence feeding. Assays were carried out with the use of non-toxic red and blue food coloring dyes (McCormick & Co., Inc., Hunt Valley, MD), after Cassill and Tschinkel (1999), mixed in sucrose solutions so that food choice could easily been seen in the ant gaster, the posterior portion of the body behind the petiole (Supplemental Fig. 2). Dyes for control and treatment solutions were switched between each replicate to control any effect of color on ant preference. Tests containing only sucrose water (no Se added) and dyes were also conducted as an added positive control to further rule out an effect of color preference on the outcome. Each Se form was tested using at least 6 replicates of 50 worker ants each for both sucrose concentrations. Choice tests were conducted for 2 h and observations were made at different time points (0,1, 2, 30, 60, 90, and 120 min) documenting the number of ants present at each treatment station. At the end of 2 h, all 50 ants were removed and placed in a freezer overnight. The dead ants were removed from the freezer and crushed between 2 pieces of filter paper to allow absorption of dye onto the paper (Supplemental Fig. 3). The number of ants containing red, blue, purple and non-colored gasters was counted blindly (observer was unaware of dye assignments for treatments) to remove any observer bias.

2.5. Statistical analysis

Analysis was conducted using R version 2.14.1 (The R Foundation for Statistical Computing 2011). All response variables were examined for normality using the Jarque–Bera Test, when samples sizes were greater than 50, and Shapiro–Wilk's Test, when samples sizes were less than 50. Tests for homogeneity of variance were conducted using the Bartlett's Test or Levene's Test. For multiple comparison testing, a one-way analysis of variance (ANOVA) was used when data assumptions were valid. When data had equal variance and could not be transformed for normality, a one-way Welch Test was used. Post hoc comparisons were conducted using Tukey's HSD for ANOVA and Games and Howell following Welch tests. The specific statistical tests used for mortality, bioaccumulation, and choice experiments are described below.

2.5.1. Mortality

Cox Proportional Hazard Regression Models were used to track survival over time between each form using the R package "survival" (Therneau, 1999). To follow up on differences in rates of mortality observed between concentrations within each Se form, multiple comparisons were conducted for time points of days 5, 7, 11 and 14 using ANOVA. For calculation of LC_{50}s, data were corrected for control mortality using Abbott's formula (Eq. (1); Abbott, 1925). Lethal concentrations for days 7 and 14 were generated following the methods described in Jeske et al. (2009) and using the R package "drc" (Ritz and Streibig, 2005).

$$\%\text{Mortality} = \frac{x+y}{x} \times 100 : \quad \begin{array}{l} x = \%\ \text{survival in untreated control} \\ y = \%\ \text{survival in treatment group} \end{array} \tag{1}$$

2.5.2. Bioaccumulation

The initial model included the factors: treatment, form of Se and the interaction of treatment and form. The interaction between form and treatment was not significant (ANOVA, $F = 0.74$, $df = 21$, $P = 0.78$) and was removed from the model; the two factors of form and treatment concentration were then analyzed separately. Because overall data could not be transformed to normality, they were analyzed using the non-parametric Kruskal–Wallis test. Comparisons of accumulated Se across treatments, within each form, were analyzed using Wilcoxon–Mann–Whitney for SeMet and selenite (data were normal), a Welch test for MeSeCys and an ANOVA for selenate. Comparisons across forms at each concentration treatment were conducted using ANOVA and post hoc Tukey's HSD. Accumulated concentrations reported here are from the combined weights (as described in the Materials and methods section) rather than from individual ants.

2.5.3. Choice tests

Data from observations of ants feeding at stations during the assay were analyzed using a generalized linear model. Based on the Akaike information criterion (AIC) value, the negative binomial (NB) model was found to have a better fit compared to the Poisson model. The final model included the number of ants present at each station as the response variable and the factors: trial (for each form of Se), color of dye, treatment, sucrose concentration, and time point during the assay. Multiple comparison tests using ANOVA or Welch tests were used to investigate ant choice by comparing the percentage of ants visiting the following options: control, treatment, both, or no stations (gathered from counting scoring colored gasters at the end of the assay). Similar analyses were carried out for assays that included only added dye to sucrose (positive controls), which found no effects for the color of dye as a possible attraction factor to one station or another.

3. Results

3.1. Mortality

Differences in mortality between ant cohorts were found to be dependent on the chemical form and concentration of Se ingested over the two-week assay (form: $\chi^2 = 1168$, $df = 2,23$, $P < 0.0001$; concentration: $\chi^2 = 192$, $df = 7,23$, $P < 0.0001$). There was also an effect of the interaction between form and concentration on ant mortality ($\chi^2 = 236$, $df = 14,23$, $P < 0.0001$). The analysis of time to significant mortality for each form showed that ants feeding on sucrose containing MeSeCys experienced significant mortality sooner, compared to all other forms (Fig. 1). For example, a comparison across treatments for MeSeCys revealed that by day 5, concentrations of 20–50 μg Se ml^{-1} produced significantly greater mortality than control and lower concentration treatments (ANOVA; $F = 13$, $df = 7,16$, $P < 0.0001$). In contrast, differences in mortality across treatments were not seen until after day 7 for all other forms of Se. It was only until day 11 that a statistically significant difference occurred between the highest treatment of selenate (54 μg Se ml^{-1}), and all other treatments (ANOVA; $F = 7.3$, $df = 6,21$, $P < 0.01$). Differences in mortality among treatments did not occur for ants feeding on sucrose with SeMet until day 14 (ANOVA; $F = 2.8$, $df = 7,24$, $P < 0.05$); no statistical difference in mortality across treatments was found for selenite for any time point.

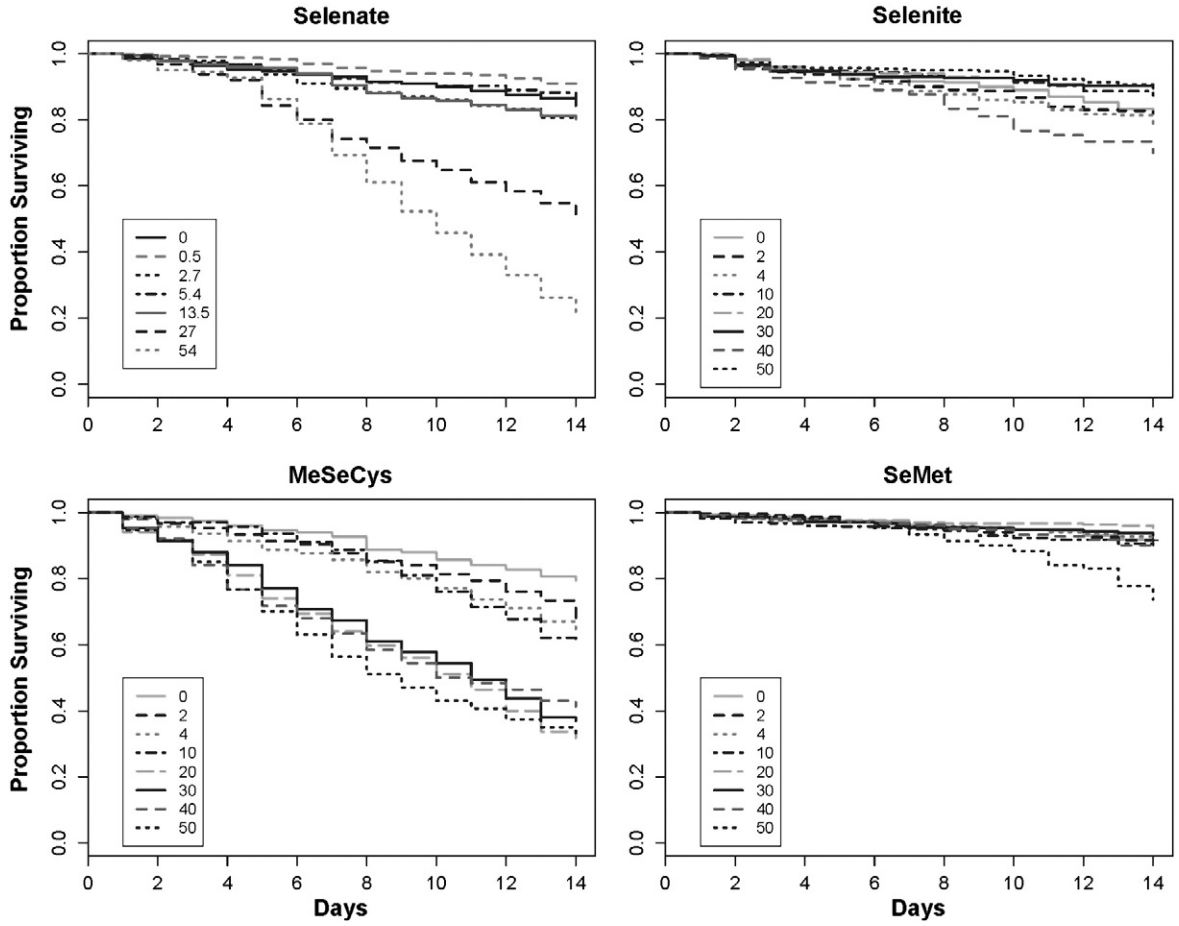

Fig. 1. Cox proportional hazard model plot displaying proportion survival/mortality of ants over 14 days following ingestion of selenate, selenite, methylselenocysteine (MeSeCys) and selenomethionine (SeMet). Legends within each graph correspond to concentrations fed in μg Se ml^{-1}.

Generation of LC_{50}s allowed for comparison of toxicity among forms and over time. Non-overlap of 95% confidence intervals indicates that for Argentine ant workers, the order of highest to lowest toxicity for the four forms tested is as follows: MeSeCys > selenate > SeMet > selenite (Table 1). Longer exposures generally decreased the LC_{50}s from day 7 to day 14. For example, approximately 88 μg ml^{-1} of MeSeCys was enough to cause 50% mortality after the first week, but the LC_{50} declined to 28 μg Se ml^{-1} after two weeks. The LC_{50}s for SeMet and selenite after the first week were not attainable at the given experimental concentrations (mortality was not high enough), however, after two weeks of chronic ingestion, LC_{50}s stabilized below 200 and 800 μg Se ml^{-1}, respectively.

Table 1
Lethal concentrations that kill 50% of the population (LC_{50}) for four selenium forms after 7 and 14 days.

Selenium form	Day	LC 50 (mg L^{-1})	95% confidence limits
Selenate	7	131.57	97.93–176.78
	14	34.8	32.48–37.29
Selenite	7	44×10^5	$3.02–6 \times 10^{12}$
	14	709.89	$134.37–3 \times 10^3$
Methylselenocysteine (MeSeCys)	7	87.83	69.15–111.55
	14	27.68	24.00–31.92
Seleno-L-methionine (SeMet)	7	29×10^3	$40.73–21 \times 10^6$
	14	176.17	97.3–318.95

3.2. Bioaccumulation

Ants from control treatments were found to contain small amounts of Se (Table 2). This was attributed to their diet of cockroaches, also found to contain very low concentrations of Se (below 1 μg Se g^{-1}), which were fed Purina Dog Chow™ that was supplemented with low concentrations of Se. Because all ants in the experiments were fed the same materials, and accumulations in the control ants were quite low and not statistically different between controls, treatment accumulations were not adjusted.

3.2.1. Between form comparisons

Surviving ants were found to contain Se body burdens at concentrations greater than they had been provided in sucrose treatments. Mean concentrations reached approximately twice as much for MeSeCys and selenate, but more than double for SeMet and selenite at all treatment concentrations (Table 2, Fig. 2). The chemical form was also found to affect the concentration of Se present in ant tissue (Kruskal–Wallis; $\chi^2 = 8.09$, df = 3, $P < 0.05$). Overall accumulation comparisons between compounds revealed that ants fed SeMet accumulated more Se than ants fed MeSeCys (Wilcoxon–Mann–Whitney, W = 388, $P < 0.05$) or selenate (Wilcoxon–Mann–Whitney, W = 301, $P < 0.05$). Ants fed different chemical forms of Se differed in accumulated Se when fed treatment concentrations of 10 μg Se ml^{-1} (ANOVA; F = 4.8, df = 3,12, $P < 0.05$), 20 μg Se ml^{-1} (ANOVA; F = 3.9, df = 3,12, $P < 0.05$), 30 μg Se ml^{-1} (ANOVA; F = 8.4, df = 3,12, $P < 0.01$) and 50 μg Se ml^{-1} (ANOVA; F = 4.2, df = 3,10, $P < 0.05$). Pairwise

Table 2
Mean, standard error and median values for accumulated selenium in ants after two weeks of chronic ingestion of one of four selenocompounds.

Conc. fed (μg Se/mL)	Selenate			Selenite			MeSeCys			SeMet		
	Mean	SEM	Median	Mean	SEM	Median	Mean	SEM	Median	Mean	SEM	Median
0	1.8	1.6	0.2	1.2	1.0	0.2	4.2	1.3	3.6	1.4	0.7	0.9
2	5.9	2.3	13.8	15.7	5.0	13.8	7.9	1.4	8.5	29.7	18.1	11.5
4	7.3	2.8	14.3	14.1	2.8	14.3	13.6	4.2	15.9	85.5	63.3	22.9
10	21.5	7.2	27.6	28.5	4.7	27.6	24.7	9.0	17.5	57.3	8.6	60.0
20	46.3	5.2	36.5	43.5	11.9	36.5	48.9	14.6	36.8	98.3	16.2	96.3
30	62.3	7.1	59.8	68.0	15.9	59.8	55.8	25.2	54.0	160.8	16.0	150.6
40	74.2	30.1	95.0	171.5	91.0	95.0	61.4	15.4	75.0	193.6	54.3	150.7
50	77.2	24.8	114.7	108.7	13.3	114.7	82.5	10.1	82.9	150.1	15.0	152.3

comparisons confirmed that ants feeding on sucrose containing SeMet accumulated greater amounts of Se than MeSeCys and selenate at 10, 30, and 50 μg ml^{-1} (Tukey's HSD, $p < 0.05$) and a greater amount than selenite at 30 μg ml^{-1} (Tukey's HSD $p < 0.05$). The highest mean accumulation (Table 2) was observed for ants fed 40 μg Se ml^{-1} as SeMet, with a mean body burden of 193 μg Se g^{-1}. Ants feeding on sucrose containing 40 μg Se ml^{-1} as selenite had a mean accumulation of 171 μg Se g^{-1}. The lowest mean accumulations of Se were seen for ants feeding on sucrose containing 2 μg Se ml^{-1} of MeSeCys or selenate, where mean concentrations in ants reached ≈8 and 6 μg Se g^{-1}, respectively.

3.2.2. Within compound comparisons

Selenium accumulated by ants was also found to differ across treatment concentrations (Kruskal–Wallis; $\chi^2 = 83$, df = 7, $P < 0.0001$), with a general trend for increased Se in ant tissue at higher treatment concentrations (Table 2). Worker ants bioaccumulated significantly more Se than control ants for all treatments when fed SeMet (Wilcoxon; $W = 0$, $P < 0.01$) and selenite (Wilcoxon; $W = 0$, $P < 0.05$). In contrast, accumulation in ants feeding on sucrose with MeSeCys only reached mean concentrations statistically greater than controls when fed the highest treatment concentration of 50 μg Se ml^{-1} (Welch test; $F = 9.1$, df = 7,8.94, $P < 0.01$; Games and Howell $P < 0.05$). A difference was also seen for ants fed sucrose containing selenate, but this was only reached at treatment concentrations ≥20 μg Se ml^{-1} (ANOVA; $F = 8.9$, df = 7,16, $P < 0.01$; Tukey's HSD, $P < 0.01$).

3.3. Choice tests

The analysis of ant visits to stations during the assays only revealed significance against the factors of "time" and "trial". Significance of time was not unexpected, as ant activity at all stations began to taper after the first hour (GLM NB; $\chi^2 = 80.2$, df = 6, $P < 0.0001$). The significance of trial indicates that ants had higher feeding activity for some trials compared to others ($\chi^2 = 87$, df = 3, $P < 0.0001$). However, because there was no statistical difference in ant visits between treatments ($\chi^2 = 0.495$, df = 1, $P = 0.482$), nor was there a significant interaction between treatments and trial ($\chi^2 = 4.3$, df = 3, $P < 0.230$), this suggests that ants fed equally from stations with and without Se regardless of the Se form provided (one form for each trial). In addition, there was neither an effect of sucrose concentration nor its interaction with the other factors, demonstrating that Se was not repellent to ants ($\chi^2 = 1.7$, df = 1, $P = 0.196$). Color was also found not to be a factor influencing ant preference, which supports earlier work showing no such effect for positive controls ($\chi^2 = 1.8$, df = 1, $P = 0.405$).

Data from gaster counts at the end of the assay, which represented overall decisions made by ants based on the final color of their gasters, supported the findings above. There was a difference detected in the percentage of ants that fed from either treatment station, both or none at all (ANOVA/Welch test; df = 3, $P < 0.001$). However, post hoc comparisons revealed this significant difference existed only for the option of no feeding (Games and Howell or Tukey HSD; df = 3, $P < 0.05$), indicating that the percentage of ants feeding from stations was greater than the percentage of ants that chose not to feed at all. Furthermore, in all cases, there was no statistically significant difference in the percentage of ants feeding at control *versus* Se-treatment stations when provided choices in 10% sucrose (MeSeCys $P = 0.999$; Selenate $P = 0.636$; SeMet $P = 0.997$; Selenite $P = 0.992$) or 30% sucrose (MeSeCys $P = 0.067$; Selenate $P = 0.192$; SeMet $P = 0.326$; Selenite $P = 0.843$). Thus, there was no evidence that the ants responded to the presence of any form of Se that was tested, regardless of the toxicity or background sucrose concentration.

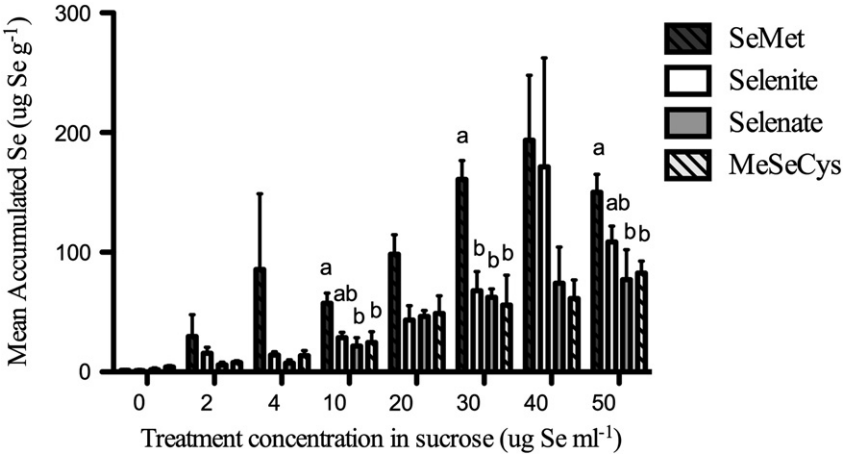

Fig. 2. Comparison of bioaccumulation between forms for accumulated selenium in ants following two-week ingestion at various concentrations. Letters represent means that are significantly different from each other within a given concentration. Differences were confirmed using ANOVA and post hoc Tukey's pairwise comparisons.

4. Discussion

Argentine ant workers experienced significant mortality within two weeks of chronically ingesting sucrose containing Se, where the rate of survival was largely dependent on the concentration and form of Se provided. The Se forms methylselenocysteine and selenate exhibited overall greater toxicity compared to selenomethionine and selenite. In addition, methylselenocysteine appears to have greater acute toxicity as evidenced by significant mortality as early as day 5, whereas all others had more of a delayed effect. This is similar to a study by Jensen et al. (2005) that found selenocysteine was the most toxic form of Se tested against a phorid fly (*Megaselia scalaris*: Diptera: Phoridae). However, these results are in contrast to other reports which found methylselenocysteine to be among the least toxic to larvae and adult honeybees (*Apis mellifera*: Hymenoptera: Apidae; Hladun et al., 2013a) and of intermediate toxicity to beet armyworms (*Spodoptera exigua*, Lepidoptera: Noctuidae; Vickerman and Trumble, 1999). Even so, the LC_{50}s reported here suggest all forms have the potential to cause substantial mortality at the ecologically-relevant ranges that have been reported in plant components (Hladun et al., 2011; Quinn et al., 2011), especially in instances where plants convert inorganic forms to their less toxic form methylselenocysteine.

Surviving workers were also found to bioaccumulate selenium for all forms of selenium fed at nearly twice the concentrations provided in sucrose across all treatments. Interestingly, ants feeding on sucrose containing the 2 forms that were found to cause lower toxicity accumulated the highest body burdens. Selenomethionine and selenite were discovered to have a delayed toxic effect, however ants feeding on sucrose containing these forms accumulated over 150 μg Se g^{-1}, whereas those feeding on the more toxic methylselenocysteine and selenate averaged of 85 μg Se g^{-1} or less. The choice tests determined that this pattern did not occur due to avoidance of any particular form of Se, so we predict that selective feeding will not play a critical role in natural settings for Argentine ants and possibly other ant species. Differences in toxicity and accumulation caused by different Se forms are likely due to differences in metabolic fate and bioavailability of each compound. It is also probable that the form present upon ingestion is different than that which is causing toxicity, if they undergo biotransformation within the worker ants. This might also help to explain the discrepancies between reports on toxicity of certain selenocompounds between invertebrates. As of now, the behavior of Se within the ant body is unclear, but identification of the particular selenocompounds within ant bodies should help to reveal the mechanisms of toxicity.

Ants were not deterred by Se in sucrose for any form, regardless of the sugar concentration. The observation that Se did not deter Argentine ants from feeding is not completely unexpected, as the literature does include reports of some insect species demonstrating no avoidance to selenium (Vickerman and Trumble, 1999; Freeman et al., 2006). Most, however, report an adverse reaction to Se in plant tissue (Vickerman and Trumble, 1999; Jensen and Trumble, 2003; Hanson et al., 2004; Galeas et al., 2008; Hladun et al., 2013b). In contrast, when nectar extracts from plant species containing possible defensive compounds, *e.g.* phenolic compounds, alkaloids, non-protein amino acids, were tested against several species of ants, results indicated predominantly non-avoidance of ants to nectar, whereas ants showed mixed results towards floral tissue extracts (Guerrant and Fiedler, 1981). Therefore, predicting the responses of invertebrate species to new and potentially toxic materials will require much more information than is currently available. Furthermore, accumulation of Se by ants, even at the lowest concentrations reported in this study, may be sufficient to pose a problem for other organisms that depend on ants as food. Given the relatively high body burdens of Se in ants in our experiments, studies on the potential for Se to be transferred from invertebrates to predatory organisms also appear warranted.

The results of this study point to several potentially detrimental implications for ant communities residing in seleniferous habitats where plant resources available to ants may contain significant concentrations of this element. If ants are undeterred by Se in their diet, workers will continue to gather and provide toxic food to other members of their colony. Studies investigating heavy metal accumulation in ants have shown that body burdens are often highest in workers due to different feeding strategies among castes or because of dilution by trophallaxis, before reaching the brood and reproductives (Grześ, 2010). Nonetheless, sub-lethal concentrations reaching immature stages may still impact larval development, as was seen for larval honeybees fed Se (Hladun et al., 2013a). Given the rate of mortality found here for ant workers, as well as most reports documenting primarily negative impacts on growth and development (Lemly, 1997; Jensen et al., 2007; Popham and Shelby, 2007; Hladun et al., 2013a), Se has the potential to impact colony size. At least for the Argentine ant, a decrease in population size may affect their ability to exploit new territory and compete against other ant species, as large numbers are often a key factor in their success (Holway and Suarez, 1999).

The effects of Se on survival for one ant species may or may not be experienced by other ant species within the disturbed habitat. For instance, Se-mediated shifts in ant species composition within a given community are possible because ants often vary in physiological mechanisms for tolerance of pollutants (Grześ, 2010). Differences in feeding strategies may also contribute to the survival of certain ant species compared to others residing in the same habitat; populations will likely be exposed to different concentrations of Se, and these may depend either on the plant species from which they are foraging and to which trophic group their diet is categorized (*e.g.* herbivore, omnivore, predator). This may allow some species to develop resistance if exposed to relatively low concentrations, whereas others may be exposed to immediately lethal concentrations. Furthermore, changes in the ant community caused by pollutants may also impact both the arthropod and plant communities in their environment, especially if the pollutant effects the survival of certain keystone species.

5. Conclusions

This is the first in depth study to investigate the toxic effect of selenium in ants. Mortality to selenium was found to be dependent on the form and concentration of selenium ingested. In addition, the findings of this study and others support the idea that toxicity to various chemical forms of selenium is also species-specific. Argentine ant workers were also capable of bioaccumulating Se; body burdens are also reliant on form and concentration consumed. Interestingly, Se does not act as a repellent to Argentine ant workers when provided in an artificial diet, regardless of the background sucrose concentration. Collectively, these findings suggest the possibility for detrimental effects of Se on individual ant species residing in recently contaminated habitats. This also has ecological implications at the community level for shifts in ant species composition, which may ultimately lead to changes in ecosystem functioning provided by sensitive keystone species. Further research is necessary to compare ant diversity in similar habitats contaminated with varying concentrations of Se. Lastly, Argentine ants used in this study were naïve to the Se concentrations provided, which suggests potential effects on their ability to invade areas with elevated Se. This is a consideration in invasive ecology, which also remains unexplored.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.02.060.

Acknowledgments

We would like to thank Dr. Dong-Hwan Choe, William Carson, Dr. Kristen Hladun, Greg Kund and Dr. Sean M. Prager for their review of this manuscript. The authors also thank Woody Smith and Kristen Hladun for their helpful assistance with the chemical analyses. We also extend our appreciation to Nichole Drew, Haylee Drew, and Kris Gilbert for their help with data collection, and to Greg Kund and Sean

Prager for the advice they offered on the statistical portion of this project. Our gratitude also goes out to Dr. Les Greenberg for his advice and assistance with ant collecting, rearing, and colony maintenance.

References

Abbott W. A method of computing the effectiveness of an insecticide. J Econ Entomol 1925;18:265–7.

Babula P, Adam V, Opatrilova R, Zehnalek J, Ladislav H, Kizek R. Uncommon heavy metals, metalloids and their plant toxicity: a review. Environ. Chem. Lett. 2008;6:189–213.

Bañuelos GS, Fakra SC, Walse SS, Marcus MA, Yang SI, Pickering IJ, et al. Selenium accumulation, distribution, and speciation in spineless prickly pear cactus: a drought- and salt-tolerant, selenium-enriched nutraceutical fruit crop for biofortified foods. Plant Physiol 2011;155:315–27.

Brown G, Foster A, Ostergren J. Mineral surfaces and bioavailability of heavy metals: a molecular-scale perspective. Proc Natl Acad Sci U S A 1999;96:3388–95.

Cassill D, Tschinkel W. Regulation of diet in the fire ant, *Solenopsis invicta*. J Insect Behav 1999;12:307–28.

Christian CE. Consequences of a biological invasion reveal the importance of mutualism for plant communities. Nature 2001;413:635–9.

Council NR. Selenium in nutrition. Revised Edition. Washington D.C.: The National Academies Press; 1983

Daniels LA. Selenium metabolism and bioavailability. Biol Trace Elem Res 1996;54:185–99.

Eeva T, Sorvari J, Koivunen V. Effects of heavy metal pollution on red wood ant (*Formica s. str.*) populations. Environ Pollut 2004;132:533–9.

Eisler R. Selenium hazards to fish, wildlife, and invertebrates: a synoptic review; 1985 [Laurel, MD].

Eisler R. Selenium. Eisler's encyclopedia of environmentally hazardous priority chemicals. Elsevier B.V.; 2007. p. 737–59.

Folgarait P. Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers Conserv 1998;7:1221–44.

Freeman JL, Quinn CF, Marcus MA, Fakra S, Pilon-Smits EAH. Selenium-tolerant diamondback moth disarms hyperaccumulator plant defense. Curr Biol 2006;16:2181–92.

Galeas ML, Klamper EM, Bennett LE, Freeman JL, Kondratieff BC, Quinn CF, et al. Selenium hyperaccumulation reduces plant arthropod loads in the field. New Phytol 2008;177:715–24.

Gómez JM, Zamora R, Hódar JA, García D. Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. Oecologia 1996;105:236–42.

Grześ IM. Ant species richness and evenness increase along a metal pollution gradient in the Bolesław zinc smelter area. Pedobiologia (Jena) 2009;53:65–73.

Grześ IM. Ants and heavy metal pollution — a review. Eur J Soil Biol 2010;46:350–5.

Guerrant EOJ, Fiedler PL. Flower defenses against nectar-pilferage by ants. Biotropica Suppl Reprod Bot 1981;13:25–33.

Hanson B, Lindblom SD, Loeffler ML, Pilon-Smits EAH. Selenium protects plants from phloem-feeding aphids due to both deterrence and toxicity. New Phytol 2004;162:655–62.

Hladun KR, Parker DR, Trumble JT. Selenium accumulation in the floral tissues of two Brassicaceae species and its impact on floral traits and plant performance. Environ Exp Bot 2011;74:90–7.

Hladun KR, Smith BH, Mustard JA, Morton RR, Trumble JT. Selenium toxicity to honey bee (*Apis mellifera* L.) pollinators: effects on behaviors and survival. PLoS One 2012;7:e34137.

Hladun KR, Kaftanoglu O, Tran KD, Trumble JT. Effects of the pollutant selenium on survival, development and accumulation in the honey bee (*Apis mellifera* L.). Environ Toxicol Chem 2013a;32(11):2584–92.

Hladun KR, Parker DR, Tran KD, Trumble JT. Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (*Raphanus sativus* L.). Environ Pollut 2013b;172:70–5.

Hoffmann DJ. Selenium. In: Hoffman DJ, editor. Handbook of ecotoxicology. Boca Raton, FL.: Lewis Publishers; 2003. p. 465–500.

Hoffmann BD, Griffiths AD, Andersen AN. Responses of ant communities to dry sulfur deposition from mining emissions in semi-arid tropical Australia, with implications for the use of functional groups. Austral Ecol 2000;25:653–63.

Holway DA, Suarez AV. Animal behavior: an essential component of invasion biology. Trends Ecol Evol 1999;14:328–30.

Jensen P, Trumble JT. Ecological consequences of bioavailability of metals and metalloids in insects. Recent Res Dev Entomol 2003;4:1–17.

Jensen PD, Rivas MD, Trumble JT. Developmental responses of a terrestrial insect detritivore, *Megaselia scalaris* (Loew) to four selenium species. Ecotoxicology 2005;14:313–22.

Jensen PD, Sorensen MA, Walton WE, Trumble JT. Lethal and sublethal responses of an aquatic insect *Culex quinquefasciatus* (Diptera: Culicidae) challenged with individual and joint exposure to dissolved sodium selenate and methylmercury chloride. Environ Toxicol 2007:287–94.

Jeske DR, Xu HK, Blessinger T, Jensen P, Trumble J. Testing for the equality of EC50 values in the presence of unequal slopes with application to toxicity of selenium types. J Agric Biol Environ Stat 2009;14:469–83.

Klotz J, Hansen L, Pospischil R, Rust M. Urban ants of North America and Europe: identification, biology, and management. Ithica, New York: Cornell University Press; 2008.

Lach L, Parr C, Abbott KIn: Lach L, Parr C, Abbott K, editors. Ant ecology. Oxford, New York: Oxford University Press; 2010.

Lemly AD. Environmental implications of excessive selenium: a review. Biomed Environ Sci 1997;10:415–35.

Losey JE, Vaughan M. The economic value of ecological services provided by insects. Bioscience 2006;56:311.

MacMahon JA, Mull JF, Crist TO. Harvester ants (*Pogonomyrmex* spp.): their community and ecosystem influences. Annu Rev Ecol Syst 2000;31:265–91.

Markin GP. Foraging behavior of the Argentine ant in a California citrus grove. J Econ Entomol 1970;63:740–4.

Mayland HF. Selenium in plant and animal nutrition. In: Frankenberger Jr W, Benson SM, editors. Selenium in the environment. New York: Marcel Dekker, Inc.; 1994. p. 29–45.

Migula P, Głowacka E. Heavy metals as stressing factors in the red wood ants (*Formica polyctena*) from industrially polluted forests. Fresenius J Anal Chem 1996;354:653–9.

Ohlendorf HM, Santolo GM. Kesterson Reservoir — past, present, and future: an ecological risk assessment. In: Frankenberger Jr WT, Benson SM, editors. Selenium in the environment. New York: Marcel Dekker, Inc.; 1994. p. 69–117.

Parker DR, Page AL. Vegetation management strategies for remediation of selenium-contaminated soils. In: Frankenberger WT, Benson SM, editors. Selenium in the environment. New York, New York: Marcel Dekker, Inc.; 1994. p. 327–42.

Perfecto I. Ants (Hymenoptera: Formicidae) as natural control agents of pests in irrigated maize in Nicaragua. J Econ Entomol 1991;84:65–70.

Petal J, Nowak E, Jakubczyk H, Czerwiński Z. Effect of ants and earthworms on soil habitat modification. Ecol Bull 1977:501–3.

Popham HJR, Shelby KS. Effect of inorganic and organic forms of selenium supplementation on development of larval *Heliothis virescens*. Entomol Exp Appl 2007;125:171–8.

Power ME, Tilman D, Estes JA, Menge BA, Bond WJ, Mills LS, et al. Challenges in the quest for keystones. Bioscience 1996;46:609–20.

Quinn CF, Prins CN, Freeman JL, Gross AM, Hantzis LJ, Reynolds RJB, et al. Selenium accumulation in flowers and its effects on pollination. New Phytol 2011;192:727–37.

Rico-Gray V, Oliveira PS. The ecology and evolution of ant–plant interactions. Chicago: The University of Chicago Press; 2007.

Ritz C, Streibig JC. Bioassay analysis using R. J Stat Softw 2005;12:1–22.

Rosumek FB, Silveira FAO, de S. Neves F, de U Barbosa NP, Diniz L, Oki Y, et al. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. Oecologia 2009;160:537–49.

Samson DA, Philippi TE, Davidson DW. Granivory and competition as determinants of annual plant diversity in the Chihuahuan desert. Oikos 1992;65:61–80.

Seiler R, Skorupa J, Peltz L. Areas susceptible to irrigation-induced selenium contamination of water and biota in the Western United States. US Geol Surv 1999;1180.

Sorvari J, Rantala LM, Rantala MJ, Hakkarainen H, Eeva T. Heavy metal pollution disturbs immune response in wild ant populations. Environ Pollut 2007;145:324–8.

Styrsky JD, Eubanks MD. Ecological consequences of interactions between ants and honeydew-producing insects. Proc R Soc B 2007;274:151–64.

Therneau TM. A package for survival analysis in S. Mayo Found; 1999.

Vanbergen AJ, Initiative IP. Threats to an ecosystem service: pressures on pollinators. Front Ecol Environ 2013;11:251–9.

Vandermeer J, Perfecto I, Nuñez G. Ants (*Azteca* sp.) as potential biological control agents in shade coffee production in Chiapas, Mexico. Agrofor Syst 2002;56:271–6.

Vickerman DB, Trumble J. Feeding preferences of *Spodoptera exigua* in response to form and concentration of selenium. Arch Insect Biochem Physiol 1999;42:64–73.

Vickerman DB, Trumble JT. Biotransfer of selenium: effects on an insect predator, *Podisus maculiventris*. Ecotoxicology 2003;12:497–504.

Vickerman DB, Shannon MC, Bañuelos GS, Grieve CM, Trumble JT. Evaluation of Atriplex lines for selenium accumulation, salt tolerance and suitability for a key agricultural insect pest. Environ Pollut 2002;120:463–73.